A STABILITY INDICATING HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF VALSARTAN AND RAMIPRIL IN BINARY COMBINATION

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ABSTRACT

A simple, sensitive and validated HPLC method has been developed to determine valsartan and ramipril simultaneously in synthetic mixture. Chromatographic separation was achieved on a C-18 column using a mixture of acetonitrile and water in the ratio 55:45 (v/v), pH adjusted to 3.6 with 88% orthophosphoric acid at a wavelength of 215 nm. Linearity of the method was found to be in the concentration range of 50-250 µg/ml for valsartan and 100-500 µg/ml for ramipril with correlation coefficient greater than 0.999. The total eluting time for the two components is less than five minutes. The method can be used for simultaneous determination of valsartan and ramipril.

Keywords: HPLC, Valsartan, Ramipril

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INTRODUCTION

Valsartan [fig 1] chemically designated as (S)-3-methyl-2-[(4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl]pentanamido]butanoic acid belong to angiotensin II receptor antagonist used for the treatment of hypertension1. A number of analytical methods have been developed for its determination in pharmaceutical formulations or in biofluids either alone or in combination with other drugs, which includes liquid chromatography-tandem mass spectrometry2, HPLC3 and a spectrophotometric analysis4.

Fig 1: Structure of Valsartan

Ramipril [fig 2], (2S,3aS,6aS)-1-[(2S)-2-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid is a prodrug which is rapidly hydrolyzed with the cleavage of an ester group through hepatic metabolism forming an active metabolite i.e., ramiprilat. This prodrug itself is a poor inhibitor of angiotensin converting enzyme (ACE) but its active metabolite has a higher affinity for ACE, thus blocking the conversion of the angiotensin I to the angiotensin II, a highly potent vasopressor activity5,6. The drug is officially listed in British Pharmacopoeia7, which describes a potentiometric titration procedure for its assay in bulk and dosage forms. The determination of ramipril along with hydrochlorothiazide in binary mixture was performed by derivative compensation technique8 as well as zero crossing derivative technique9,10. Few visible spectrophotometric11-13 and HPLC methods14,15 have also been reported for the assay of this drug in commercial dosage forms.

According to the information collected from literature there is no method reported for the simultaneous determination of valsartan and ramipril. In the present work we are therefore focused to achieve the optimum chromatographic conditions for the simultaneous determination of valsartan and ramipril in synthetic mixture.

We describe a simple, sensitive and validated HPLC method with total run time less than ten minutes for the simultaneous determination of valsartan and ramipril. The developed method can be applied successfully to quality control and for other analytical purposes.

1. Chemicals and Reagents

Valsartan and ramipril reference substance with claimed purity of 99.6% and 99.72% respectively were obtained from Madras Pharmaceuticals, Chennai. Acetonitrile (HPLC grade) and orthophosphoric acid (Analytical reagent grade) were purchased from Merck (Mumbai, India). All excipients used were of pharmaceutical grade. Water used was prepared in the laboratory using Milli-Q system (Millipore, USA).

2. Apparatus and Chromatographic Conditions

HPLC apparatus consisting of Shimadzu LC-10A system equipped with LC-10 AT dual pump, an SPD 10A variable wavelength detector (set at 215 nm), a spincobiotech software and rheodyne injection valve with a 20 µl loop was used for development and evaluation of this method. A Hypersil C18 column (250 × 4.6 mm id, 5 µm particle size) was selected. The mobile phase was composed of a mixture of acetonitrile and water in the ratio 55:45 (v/v) and the pH adjusted to 3.6 with 88% ortho phosphoric acid. The flow rate was 1 ml/min and the system was operated at room temperature 25±2°C.

3. Preparation of standard solution

A stock solution of valsartan and ramipril was prepared at about 0.5 mg/ml and 1 mg/ml respectively in mobile phase.

4. Linearity

Linearity of the proposed method was checked by analyzing five solutions in the range of 50-250 µg/ml for valsartan and 100-500 µg/ml for ramipril. Each level was prepared in triplicate.

5. Accuracy

Method accuracy was performed by adding known amounts of valsartan
and ramipril to the pre-analysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 50, 100 and 150% of the nominal analytical concentration (100 µg/ml for Valsartan and 200 µg/ml for Ramipril). Each level was prepared in triplicate.

6. Selectivity

The selectivity of the proposed method was checked by making a synthetic mixture of both the analytes with commonly occurring excipients that are found in most tablet formulations and then comparing the chromatogram with the chromatogram of the reference standard. Synthetic mixture containing 100 mg of ramipril, 50 mg of valsartan and 20 mg of each of starch, lactose, magnesium stearate and talc which are present as excipients in the pharmaceutical formulation were accurately weighed and transferred in to a 100 ml volumetric flask. The mixture was shaken well with 70ml mobile phase and then the volume was made with mobile phase and filtered. About 1 ml of the filtrate was transferred in to 10 ml volumetric flask and mobile phase was then added to volume to obtain a final concentration containing 100 µg/ml ramipril and 50 µg/ml valsartan.

7. Robustness

Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition and flow rate of the mobile phase and the detection wavelength. The chromatographic parameters of each analyte such as retention time, tailing factor, and number of theoretical plates were measured at each changed condition.

8. Precision

For evaluating the within-day precision, results of five replicate analyses of three different concentrations of samples were calculated on a single day. The between-day precision was calculated from the same samples analyzed on six different days.

9. LOD and LOQ

For calculating the LOD and LOQ values, solutions with known concentrations of analytes were injected into the HPLC system. The limit of detection (LOD) and limit of quantification (LOQ) were then measured by calculating the minimum level at which the analytes can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1) with accuracy respectively.

10. Forced Degradation studies:

Drugs were allowed to hydrolyze in base (0.1M NaOH), acid (0.1M HCl) and different strengths of hydrogen peroxide (3%, 5% and 10%). Drugs were also studied for its thermal degradation at 80°C and 105°C. For calculating the LOD and LOQ values, solutions with known concentrations of analytes were injected into the HPLC system. The limit of detection (LOD) and limit of quantification (LOQ) were then measured by calculating the minimum level at which the analytes can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1) with accuracy respectively.

The results of stability studies are presented in Table 4 and Fig 4. In this study, the LOD was found to be 0.0126 µg/ml and 0.0280 µg/ml for ramipril and valsartan respectively. The LOQ was found to be 0.0383 µg/ml and 0.0849 µg/ml for ramipril and valsartan respectively. The recovery and relative standard deviation for each of the analytes are given in Table 1.

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The acid and base samples were neutralized to pH 7. The thermal and photo degradation sample and hydrogen peroxide samples were used as such. All the samples were further diluted with mobile phase to get a concentration of 100 µg/ml of ramipril and 50 µg/ml. Blank was also treated in the same way.

The most appropriate mobile phase composition was thus found to be acetonitrile and water in the ratio of 55:45 (v/v) and pH adjusted to 3.6 with 88% of orthophosphoric acid. Under the described experimental conditions, peaks that belong to ramipril and valsartan were obtained at retention times of 1.91 and 4.84 min respectively as shown in Fig 3.

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Table 3a: Robustness Study of Valsartan.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>Retention time (min)</th>
<th>Tailing Factor</th>
<th>Theoretical Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ramipril</td>
<td>Valsartan</td>
<td>Ramipril</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>1</td>
<td>2.40</td>
<td>6.05</td>
<td>1.2</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>1.91</td>
<td>4.84</td>
<td>1.1</td>
</tr>
<tr>
<td>0.8</td>
<td>-1</td>
<td>1.62</td>
<td>4.02</td>
<td>1.3</td>
</tr>
<tr>
<td>Mobile phase Ratio (ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 : 43</td>
<td>2</td>
<td>1.92</td>
<td>5.48</td>
<td>1.1</td>
</tr>
<tr>
<td>55 : 45</td>
<td>0</td>
<td>1.91</td>
<td>4.84</td>
<td>1.1</td>
</tr>
<tr>
<td>53 : 47</td>
<td>-2</td>
<td>1.91</td>
<td>4.48</td>
<td>1.1</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>5</td>
<td>1.93</td>
<td>4.82</td>
<td>1.1</td>
</tr>
<tr>
<td>215</td>
<td>0</td>
<td>1.91</td>
<td>4.84</td>
<td>1.1</td>
</tr>
<tr>
<td>210</td>
<td>-5</td>
<td>1.93</td>
<td>4.82</td>
<td>1.1</td>
</tr>
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</table>

Table 3b: The System Suitability Data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ramipril</th>
<th>Valsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time (min)</td>
<td>1.91</td>
<td>4.82</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Number of theoretical plates/meter</td>
<td>5237</td>
<td>32474</td>
</tr>
<tr>
<td>Resolution</td>
<td>3.917</td>
<td></td>
</tr>
</tbody>
</table>

During the study it was observed that upon treatment of ramipril and valsartan with base (0.1M NaOH), acid (0.1M HCl) and different strengths of hydrogen peroxide (30%, 3%, 1%) the degradation was observed in acid, whereas no degradation was observed with different strengths of hydrogen peroxide. Table 4 indicates the extent of degradation of both the drugs under various stress conditions. Figure 4a to f and Figure 5a to e shows the chromatograms of forced degraded samples. Further it is important to note that from the chromatograms (Figs. 4a to f and Figs. 5a to e), it is evident that although the degrade peaks are observed, under the applied stress conditions like acid and oxidative degradation states. The drug is stable under alkali and other stress conditions.

Table 4: Results of Forced Degradation Studies

<table>
<thead>
<tr>
<th>S.No</th>
<th>Type of degradation</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ramipril</td>
</tr>
<tr>
<td>1</td>
<td>Alkali (0.1M NaOH, 40°C, 12hrs)</td>
<td>97.95</td>
</tr>
<tr>
<td>2</td>
<td>Acid (0.1 M HCl, 40°C,12hrs)</td>
<td>33.41</td>
</tr>
<tr>
<td>3</td>
<td>Oxidation (30% H₂O₂, 25°C 12hrs)</td>
<td>80.32</td>
</tr>
<tr>
<td>4</td>
<td>UV (24 hrs)</td>
<td>99.08</td>
</tr>
<tr>
<td>5</td>
<td>Oven (105°C, 24 hrs)</td>
<td>99.10</td>
</tr>
<tr>
<td>6</td>
<td>Sunlight (24 hrs)</td>
<td>93.57</td>
</tr>
</tbody>
</table>

Fig 4: Chromatogram of Ramipril a) Unstressed Condition b) Alkali stress, c) Acid Stress d) Oxidative degradation (30% H₂O₂) e) Thermal degradation, f) Sunlight.
CONCLUSION

A simple and accurate reverse phase HPLC method has been developed for the simultaneous determination of ramipril and valsartan. The method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation, selectivity and robustness. The run time of less than ten minutes allows its application for the routine determination of ramipril and valsartan. Further, the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. Even though no attempt was made to identify the degraded products, proposed method can be used as a stability indicating method for assay of ramipril and valsartan in combined dosage forms.

ACKNOWLEDGEMENT

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REFERENCES