Determination of Losartan Potassium and Perindopril Erbumine in Tablet Formulations by Reversed-Phase HPLC

Ankit. B. Chaudhary1*, Rakesh. K. patel2, Sunita. A. Chaudhary1

1Saraswati Institute of Pharmaceutical science, Dhanap, Gandhinagar, 382355, Gujarat, India
2Shree S. K. Patel College of pharmaceutical education and research, Ganapat University, Mehsana, Gujarat, India.

*Corres. Author: ankit_mqa@yahoo.co.in, Telephone number: 91-079-23273111 Fax number: 91-079-23273111

Abstract: The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of losartan potassium and Perindopril Erbumine in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (250 mm x 4.6 mm i.d., 5 μm particle size) column with mobile phase containing Acetonitrile: Acidic Water pH 3.4 (50+50). The flow rate was 1.5 mL/min and the eluent was monitored at 218 nm. The selected chromatographic conditions were found to effectively separate Losartan Potassium (RT- 4.57 min) and Perindopril Erbumine (RT- 2.31 min). Linearity for losartan potassium and Perindopril Erbumine were found in the range of 1-30 μg/mL. The values obtained of LODs were 0.182 and 0.272 μg/mL, LOQs were 0.551 and 0.826 μg/mL for losartan potassium and Perindopril Erbumine, respectively. The proposed method was found to be fast, accurate, precise, and reproducible and can be used for simultaneous analysis of these drugs in tablet formulations.

Keywords: Losartan potassium, Perindopril erbumine, Reversed-phase HPLC

INTRODUCTION

Losartan potassium (LOS) a potassium salt of 2-Butyl-4-chloro-1-[[2-((1H-tetrazol-5-yl) [1,1-biphenyl]-4-yl)methyl]-1H-imidazole-5-methanol, represents the first of a new class of orally active non-peptide angiotensin II (Type AT1) receptor antagonists employed in the management of essential hypertension [1,2]. The individual determination of losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography [3-5] in bulk and solid dosage forms by colorimetric method [6], in plasma by HPLC [7], simultaneously with its degradates in stressed tablets by LC-MS/MS [8] and HPTLC [9], with its active metabolite in biological fluids by HPLC [10-12] and in urine by gas chromatography-mass spectrometry [13]. Perindopril erbumine(PER), (2S, 3(infinity) S, 7(infinity) S)-1-[(S)-N-[(S)-1-carboxy- butyl] alanyl] hexahydro-2- indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), belongs to a group called angiotensin converting enzyme (ACE) inhibitors[14]. Literature survey revealed that few analytical methods have been reported for the estimation of PER include immunoassay [15], spectrophotometric [16-17], HPLC [18], biosensor method [19-21], LC–MS/MS [22- 23], capillary gas chromatographic method [24] The analytical method for simultaneous determination of LOS and PER in pharmaceutical formulation has not been reported so far. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablets.
EXPERIMENTAL

Apparatus
A Shimadzu (Columbia, MD) RP-HPLC instrument (LC-10AT vp) equipped with a photodiode array detector, manual injector with 20 μL loop, and Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5 μm particle size) and Class-VP software were used. Sartorius CP224S analytical balance (Gottingen, Germany), and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the study.

Reagents and Materials
Losartan Potassium and Perindopril Erbumide were received as a gift sample from Torrent Research Centre, Ahmedabad, Acetonitrile of HPLC grade, Glacial acetic acid of AR grade. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 μm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India). Pharmaceutical formulation of LOS and PER were purchased from local pharmacy.

Chromatographic Conditions
Phenomenex C18 column (250 mm × 4.6 mm i.d., 5 μm particle size) was used at ambient temperature. The mobile phase consisted of Acetonitrile: Acidic Water pH 3.4 (50+50) at a flow rate of 1.5 mL/min. The mobile phase was filtered through a nylon 0.45 μm-47 mm membrane filter and degassed before use. The elution was monitored at 218 nm, and the injection volume was 20 μL.

Preparation of solutions
Acidic water
Glacial acetic acid (2 mL) was dissolved in 1000 mL of water (pH : 3.4).
Mobile phase
Acidic water and acetonitrile in the ratio of 50:50 was used, sonicated and filtered through 0.45 μm filter.

LOS and PER Standard Stock Solutions
For HPLC analysis 5 mg of LOS and PER powder was weighed accurately using sartorius precision balance (readability 0.01 mg) and transferred in to 50 mL volumetric flask, dissolved and diluted to 50 mL with mobile phase to produce stock solution containing 100 μg/mL of LOS and PER respectively.

Sample Solutions
20 tablets were taken and powdered. Weighed accurately a quantity of the powder equivalent to about 50 mg of LOS and 4 mg of PER into 100 mL volumetric flask and diluted to 100 mL with mobile phase. This solution is sonicated for 20 minutes. The solution was filtered through whatman filter paper No. 41. Transfer 1 mL of solution into 10 mL volumetric flask and dilute to the mark with mobile phase. Transfer 5 mL of solution into 10 mL volumetric flask and dilute to the mark with mobile phase to get a final concentration 25 μg/mL of LOS and 2μg/mL of PER.

Determination of wavelength of maximum absorbance
The standard solutions of LOS and PER were scanned in the range of 200 –400 nm against mobile phase as a blank. LOS and PER showed maximum absorbance at 218 nm. So the wavelength selected for the determination of LOS and PER was 218 nm.

Method Validation
Calibration curve (Linearity)
A calibration curves were plotted over a concentration range of 1-30 μg/mL for LOS and PER. Accurately measured standard stock solutions of LOS and PER (0.1, 0.5, 1, 1.5, 2, 2.5 and 3 mL) were transferred to a series of 10 mL volumetric flasks and the volume in each flask was adjusted to 10 mL with mobile phase. The resulting solutions were injected into the column and the peak area obtained at flow rate 1.5 mL/min for LOS and PER respectively. Calibration curves were constructed for LOS and PER by plotting peak area
versus concentration at 218 nm. Each reading was average of three determinations.

**Accuracy (% Recovery)**
Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (LOS 10 μg/mL and PER 4 μg/mL) with three different concentrations of standards (LOS 2.4, 6 μg/mL and PER 2.4, 6 μg/mL). The good recoveries with the standard addition method (Table 3) prove the good accuracy of the proposed methods.

**Method Precision:**
For evaluation of precision, repeatability of the results for a concentration of 10 μg/mL was evaluated by 6 replicate determinations. For evaluation of intermediate precision, the results over the concentration range 1 - 30 μg/mL was evaluated by 4 replicate determinations to estimate intraday variation and another replicate determination on different 4 days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated.

**Limit of Detection and Limit of Quantification**
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S} \\
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where \(\sigma\) = the standard deviation of the response and \(S\) = Slope of calibration curve.

**Analysis LOS and PER in Combined Dosage Forms**
Pharmaceutical formulation of LOS and PER was purchased from local pharmacy. The responses of formulations were measured at 218 nm for quantification of LOS and PER by using RP-HPLC. The amounts of LOS and PER present in sample solution were determined by fitting the responses into the regression equation for LOS and PER in both the methods. Results are given in Table 4.

**RESULT AND DISCUSSION**
To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitrile: Acidic Water at pH 3.2 in (50+50) ratio and 1.5 mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 218 nm at which much better detector responses for both drugs were obtained. As it was shown in Fig. 3 the retention times were 2.31 min for PER and 4.57 min for LOS. The calibration graphs for LOS and PER were constructed by plotting the peak area versus their corresponding concentrations, good linearity for both was found over the range 1-30 μg/mL. Results obtained by applying the RP-HPLC method showed that the concentrations of LOS and PER can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of LOS and PER in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives present do not interfere with analysis of the studied mixtures. System suitability test parameters for LOS and PER for the RP-HPLC method are reported in Table 1. The optical and regression characteristics and validation parameters are reported in Table 2.

**CONCLUSION**
All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of LOS and PER in bulk and in pharmaceutical formulations without interference and with good sensitivity.

<table>
<thead>
<tr>
<th>System Suitability Parameters</th>
<th>LOS</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time</td>
<td>4.57</td>
<td>2.31</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.18</td>
<td>1.26</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>3460</td>
<td>2531</td>
</tr>
</tbody>
</table>
Table 2: Optical and Regression characteristics and validation parameters of HPLC method for analysis of LOS and PER

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LOS (µg/mL)</th>
<th>PER (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range</td>
<td>1 - 30</td>
<td>1 - 30</td>
</tr>
<tr>
<td>Detection limit</td>
<td>0.182</td>
<td>0.272</td>
</tr>
<tr>
<td>Quantitation limit</td>
<td>0.551</td>
<td>0.826</td>
</tr>
<tr>
<td>Slope</td>
<td>73934</td>
<td>18207</td>
</tr>
<tr>
<td>Intercept</td>
<td>7449</td>
<td>-841</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
<td>0.9995</td>
</tr>
<tr>
<td>Intraday RSD, %</td>
<td>0.31 - 1.05</td>
<td>0.42 - 1.87</td>
</tr>
<tr>
<td>Interday RSD, %</td>
<td>0.36 - 1.60</td>
<td>0.39 - 1.75</td>
</tr>
</tbody>
</table>

*a Intraday and interday relative standard deviation (RSD) values of the whole concentration range

Table 3: Data of recovery study for LOS and PER by HPLC method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (µg/ml)</th>
<th>Amount added (µg/ml)</th>
<th>Amount found (µg/ml)</th>
<th>% Recovery ± S.D. (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS</td>
<td>10</td>
<td>2</td>
<td>12.21</td>
<td>101.75 ± 0.6438</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>13.85</td>
<td>98.92 ± 0.4749</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>15.61</td>
<td>97.56 ± 0.8126</td>
</tr>
<tr>
<td>PER</td>
<td>4</td>
<td>2</td>
<td>6.13</td>
<td>102.16 ± 0.7347</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>8.13</td>
<td>101.62 ± 0.3426</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>10.09</td>
<td>100.92 ± 0.3392</td>
</tr>
</tbody>
</table>

Table 4: Application of the proposed method to the pharmaceutical dosage forms

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LOS</th>
<th></th>
<th>PER</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount labeled (mg)</td>
<td>Amount found (mg)</td>
<td>% Amount Found S.D. (n=3)</td>
<td>Amount labeled (mg)</td>
</tr>
<tr>
<td>Brand I</td>
<td>50</td>
<td>50.46</td>
<td>102.92 ± 1.03</td>
<td>4</td>
</tr>
<tr>
<td>Brand II</td>
<td>50</td>
<td>49.32</td>
<td>98.64 ± 1.54</td>
<td>4</td>
</tr>
<tr>
<td>Brand III</td>
<td>50</td>
<td>49.12</td>
<td>99.24 ± 1.25</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 3. A typical RP-HPLC chromatogram LOS (10 µg/ml) and PER (10µg/ml) with corresponding retention time
ACKNOWLEDGEMENT
The authors are thankful to Torrent Research Center for providing samples of pure drug. The authors are also thankful to Principal of Shree S. K. Patel College of pharmaceutical education and research for giving permission to work on HPLC.

REFERENCES
5) Kathiresan, K., Gohtandaraman, S., Manickam, M.S., MathanKumar, S., Manavalan, R., analytical method development and validation of losartan potassium tablet by rp-hplc, Rasayan J. Chem. 2008, 1, 521-525.


*****