Development and validation for determination of lisinopril dihydrate in bulk drug and formulation using RP-HPLC method

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ABSTRACT
A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for Lisinopril in bulk drug and formulation. A column having 150 × 4.6 mm in isocratic mode with mobile phase containing acetonitrile: phosphate buffer (70:30; adjusted to pH 3.0) was used. The flow rate was 0.8 ml/min and effluent was monitored at 216 nm. The retention time (min) and linearity range (µg/ml) for Lisinopril was (1.510) and (10-35). The developed method was found to be accurate, precise and selective for determination of Lisinopril in bulk and formulation.

INTRODUCTION
Lisinopril (Figure 1) is an orally bioavailable, long-acting angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity. Lisinopril, a synthetic peptide derivative, specifically and competitively inhibits ACE, which results in a decrease in the production of the potent vasoconstrictor angiotensin II and, so, diminished vasopressor activity. In addition, angiotensin II-stimulated aldosterone secretion by the adrenal cortex is decreased which results in a decrease in sodium and water retention and an increase in serum potassium [1–3]. Literature survey reveals the availability of several methods by using various Mobile phases but no method was available on this Mobile phase that is acetonitrile: phosphate buffer (70:30; adjusted to pH 3.0) which was a unique method with better results [4–6].

MATERIALS AND METHODS
Chemicals and reagents
The reference sample of Lisinopril was supplied by wockhardt Pharmaceutical Industries Ltd., Aurangabad. HPLC grade water and acetonitrile were purchased from Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from, Research Lab (India) Ltd [7, 8].

Figure 1: Structure of Lisinopril dihydrate

Chromatographic conditions
The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C18 column (150mmx4.6mm; 5µm), a 2695
### Table 1: Calibration data of lisinopril

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Mean peak area (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>742315</td>
</tr>
<tr>
<td>15</td>
<td>1419822</td>
</tr>
<tr>
<td>20</td>
<td>2121436</td>
</tr>
<tr>
<td>25</td>
<td>2810895</td>
</tr>
<tr>
<td>30</td>
<td>3531268</td>
</tr>
<tr>
<td>35</td>
<td>4265201</td>
</tr>
</tbody>
</table>

### Table 2: Precision studies for lisinopril

<table>
<thead>
<tr>
<th>Concentration of lisinopril (10µg/ml)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day</td>
</tr>
<tr>
<td>Injection-1</td>
<td>1021546</td>
</tr>
<tr>
<td>Injection-2</td>
<td>1020125</td>
</tr>
<tr>
<td>Injection-3</td>
<td>1030560</td>
</tr>
<tr>
<td>Injection-4</td>
<td>1019832</td>
</tr>
<tr>
<td>Injection-5</td>
<td>1047695</td>
</tr>
<tr>
<td>Injection-6</td>
<td>1021346</td>
</tr>
<tr>
<td>Average</td>
<td>1026851</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>10965.24</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.067852</td>
</tr>
</tbody>
</table>

### Table 3: Accuracy studies for lisinopril

<table>
<thead>
<tr>
<th>% Concentration</th>
<th>Amount added (mg)</th>
<th>Amount found (mg)</th>
<th>% Recovery</th>
<th>% Mean recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>18</td>
<td>17.95</td>
<td>99.72</td>
<td>99.64</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>19.80</td>
<td>99.00</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>22</td>
<td>22.05</td>
<td>100.22</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: System suitability parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result of lisinopril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (µg/ml)</td>
<td>10-35</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.6</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.010024</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.030374</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
<tr>
<td>Precision</td>
<td>Intra-day 1.067852</td>
</tr>
<tr>
<td></td>
<td>Inter-day 1.183517</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.64</td>
</tr>
</tbody>
</table>
binary pump, a 20μl injection loop and a 2487 dual absorbance detector and running on Waters Empower software. The UV spectrum of the drugs was taken using a shimadzu 1800 UV/VIS double beam spectrophotometer.

**Preparation of phosphate buffer (pH 3.0)**

7 gm of KH₂PO₄ was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water and pH adjusted to 3.0 with orthophosphoric acid.

**Preparation of mobile phase and diluents**

300 ml of the phosphate buffer was mixed with 700ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 μ filter under vacuum.

**Procedure**

A mixture of buffer and acetonitrile in the ratio of 30:70 v/v was found to be the most suitable mobile phase for lisinopril. The solvent mixture was filtered through a 0.45 μ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.8 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 900 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 216 nm. The run time was set at 9 min. Under these optimized chromatographic conditions the retention time obtained for the drugs lisinopril was 1.510 min [9, 10].

**Calibration plot**

About 100 mg of lisinopril was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 50 ml of a 30:70 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the solvent to get a 1000 μg/ml solution. From this, a working standard solution of the drugs (10μg/ml for lisinopril) was prepared by diluting the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 10-35 μg/ml for lisinopril was prepared from the solution in 10ml volumetric flasks using the above diluents. 20μl of each dilution was injected six times into the column at a flow rate of 0.8 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration curve constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 10-35μg/ml for lisinopril. The relevant data are furnished in Table 1 and Typical Chromatogram was shown in Figures 2,
3 and 4. The regression equations of this curves was
computed.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit
detection, limit of quantification, robustness and
system suitability parameters were studied system-
atically to validate the proposed HPLC method for
the determination of lisinopril. Solution contain-
ing 10μg/ml for lisinopril was subjected to the pro-
posed HPLC analysis to check intra-day and inter-
day variation of the method and the results are fur-
nished in Table 2. The accuracy of the HPLC method
was assessed by analyzing solutions of lisinopril
at 80%, 100% and 120% concentrated levels by
the proposed method. The results are furnished in
Table 3. The system suitability parameters are given
in Table 4.

Linearity

LOD and LOQ studies of lisinopril

The limit of detection and limit of quantification for
lisinopril was found to be 0.0101 and 0.0303 respec-
tively, which indicate the sensitivity of the method.

Specificity studies of lisinopril

The specificity of the method was ascertained by
analyzing standard drug and sample. The spot for
lisinopril in sample was confirmed by comparing the
RF and spectra of the spots with that of standards
indicating no interference of any another peak of
mobile phase, impurity.

Precision studies for lisinopril

Precision of the method was performed by intra-day
and inter-day studies. The % RSD values obtained
from peak area for lisinopril was 1.067852 intra-day
and 1.183517 inter-day. The developed method was
found to be precise as the RSD values for repeata-
bility and inter-day precision studies were <2%,
respectively, as recommended by ICH guidelines and
Shown in the Table 2.

Estimation of lisinopril in tablet dosage forms

Commercial brand of tablets was chosen for test-
ing the suitability of the proposed method to esti-
mate lisinopril in tablet formulations. Twenty
tablets were weighed and powdered. An accurately
weighed portion of this powder equivalent to 100
mg of lisinopril was transferred into a 100 ml vol-
umetric flask and dissolved in 25 ml of a 30:70 v/v
mixture of phosphate buffer and acetonitrile. The
contents of the flask were sonicated for 15 min and
a further 25 ml of the diluent was added, the flask
was shaken continuously for 15 min to ensure com-
plete solubility of the drug. The volume was made-
up with the diluent and the solution was filtered
through a 0.45 μ membrane filter. This solution
was further diluted to get the required concentra-
tions. The solution containing 10μg/ml of lisinopril
was injected into the column six times. The average
peak area of the drug was computed from the chro-
matograms and the amount of the drug present in
the tablet dosage form was calculated by using the
regression equation obtained for the pure drug [11–
13].

Accuracy studies for lisinopril

Accuracy of the method was obtained by performing
recovery studies by the standard addition method at
different levels of standard drug i.e. 80%, 100% and
120% of lisinopril to analyzed tablet powder sam-
ple and mixture were reanalyzed by the proposed
method. From the amount of drug found percent-
age recovery was calculated. The relevant results
are furnished in Table 3.

RESULTS AND DISCUSSION

In the proposed method, the retention time of lison-
opril was found to be 1.510 min. Quantification was
linear in the concentration range of 10- 35μg/ml for
lisinopril. The regression equation of the linearity
plot of concentration of lisinopril over its peak area
was found to be y = 14079x - 68594 (r^2=0.9990)
for lisinopril, where X is the concentration of lison-
opril (μg/ml) and Y is the corresponding peak area.
The limit of detection and limit of quantification for
lisinopril was found to be 0.010024l and 0.030374
respectively, which indicate the sensitivity of the
method. The use of phosphate buffer and acetoni-
trile in the ratio of 30:70 v/v resulted in peak with
good shape and resolution. The high percentage
of recovery indicates that the proposed method is
highly accurate. No interfering peaks were found in
the chromatogram of the formulation within the run
time indicating that excipients used in tablet formul-
ations did not interfere with the estimation of the
drug by the proposed HPLC method. Figure 5 shows
typical chromatogram of lisinopril. All the parameter
result of lisinopril was shown in Table 4.

CONCLUSION

The proposed HPLC method is rapid, sensitive, pre-
cise and accurate for the determination of lisinopril
can be reliably adopted for routine quality control
analysis of lisinopril bulk and in its tablet dosage
forms.

Conflict of Interest

The authors declare that they have no conflict of
interest for this study.
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REFERENCES


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