1. Introduction

Lisinopril Dihydrate (LID), (2S)-1-[(2S)-6-Amino-2-
[[(1S)-1-carboxy-3-phenyl propyl] amino] hexanoyl]
pyrrolidine-2-carboxylic acid dihydrate (Scheme1.), is
an angiotensin converting enzyme (ACE) inhibitor. It is
used in the treatment of hypertension and heart failure
(Martindale, 1999). The analytical methods reported for
the determination of LID in tablets are generally based
on spectrophotometric measurements (Asad et al., 2005;
El-Gindy et al., 2001; El-Gindy et al., 2000; El-Gindy et al.,
2001; El Yazdi et al., 1999; Paraskevas et al., 2002;
Pnzade and Mahadix., 1999; Prasad et al., 1999). Other
techniques such as liquid chromatography (El-Gindy et al.,
2001; Barbato et al., 1994; Bonazzi et al., 1997), gas
cchromatography (Avadhanulu et al., 1993) spectrofluori-
mety (El-Gindy et al., 2001; El-Yazbi et al., 1999; Esra
et al., 2003; Constantinos et al., 2004) and polarography
(Adel Razak et al., 2003; El-Enany et al., 2003), have
also been used for the same purpose. The official methods
for the assay of LID in the pure form and tablets are
potentiometric acid base titration (British Pharmacopoeia,
2003) and high performance liquid chromatography
(United States Pharmacopoeia., 2000). On the other hand
more sensitive fluoroimmunoassay (Yuan and Gilbert.,
1996), radioimmunoassay (Worland and Jarrott, 1986)
and GC with mass detection (Leis et al., 1998) methods
have been proposed for the pharmacological and phar-
macokinetic studies of the drug. However, most of these
methods are costly, tedious, and time consuming. There-
fore, the need for a rapid, economical, and selective
method is obvious, especially for a routine quality control
analysis of pharmaceutical products containing LID. The aim of this study was to develop a fast, sensitive, economical, and easy UV-Visible spectrophotometric method for the determination of LID in raw and pharmaceutical formulations. 1,2-Naphthoquinone-4-sulphonate (NQS) has been used for the determination of many pharmaceutical amines (Gallo-Martinez et al., 1998; Wang et al., 2004; Darwish, 2005). The reaction between NQS and LID has not been investigated yet. Therefore, the present study was devoted to explore NQS as a derivatizing reagent in the development of selective and sensitive spectrophotometric method for the determination of LID in tablets.

2. Experimental

2.1. Apparatus

All of the spectrophotometric measurements were made with a Double beam UV-1800 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells (SHIMADZU Japan), temperature controller was used for the spectrophotometer measurements, pH meter model pH 211(HANNA Italy) was used for adjusting pH.

2.2. Chemicals and materials

Lisinopril dihydrate (LID; AMIPHARMA Laboratories Ltd, Khartoum, Sudan) was obtained and used as received; its purity was 100.2±1.25%. 1,2-naphthoquinone-4-sulfonate; (NQS; Aldrich chemical Co., St. Louis, USA). Lisinopril tablets (Pharma international; Jordan) are labeled to contain 5mg of LID tablet. All reagents were of analytical grade reagent. Double-distilled water was used in all experiments.

2.3. Preparation of standard and sample solutions

2.3.1. Stock standard solution of Lisinopril dihydrate (1000 µg/mL)

An accurately weighed 0.10g standard sample of LID was dissolved in water, transferred into a 100mL standard flask and diluted to the mark with water and mixed well. The solution was stable for at least two weeks at 4ºC.

2.3.2. Sodium 1,2-naphthoquinone-4-sulfonic (NQS) solution 0.3% (w/v)

A weighed 0.30 g NQS was dissolved in water transferred into a 100 mL standard flask and diluted to the mark with water and mixed well. The solution was freshly prepared and protected from light.

2.3.3. Buffer solutions

Buffer solution of pH 12.0 was prepared by mixing 25 mL of 0.2 M KCl with 12 mL of 0.2 M NaOH in 100 mL volumetric flask and adjusted by a pH meter. Buffer solutions of different pH value were also prepared according to literature method.

2.3.4. Tablets sample solution

Twenty tablets were weighed accurately and pulverized. Then an accurately weighed amount equivalent to 100 mg was transferred into a 100 mL calibrated flask, and dissolved in about 40 mL in double distilled water for LID, after 10 minutes of continuous shaking, was filtered into a 100 ml of calibrated volumetric flask through Whatmann no:41 filter paper and was diluted to 100 ml, to obtain a stock solution with a concentration of 1000 µg/mL. The prepared solution was diluted quantitatively to obtain a suitable concentration for the analysis, and then the general procedure was followed as described below.

2.4. General recommended procedure

A 1.0 mL of 100 mg/L LID was transferred into A 10.0 mL standard flask 1.5 mL NaOH-KCl buffer solution of pH 12.00, 1.00 mL of 0.3% NQS were added sequentially, diluted to the mark with water and mixed well. This solution stood for 5 min at room temperature. The absorbance of the solution was measured at 481 nm against blank reagent prepared with the same reagent concentration, except the drug.

2.5. Composition of product

The Job's method of continuous variation was employed (Job P., 1964). Equimolar (2.5x10⁻³ M) aqueous solutions of LID and NQS were prepared. Series of 10-mL portions of the master solutions of LID and NQS were made up comprising different complementary proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0). The solution was further treated as described under the general recommended procedures.

3. Result and discussion

3.1. Absorption spectra

According to the procedure, the absorption spectrum of the product I produced by the reaction between LID
and NQS is recorded Figure 1. As can be seen in Figure 1, the maximum absorption wavelength of product (I) is at 481 nm, and the $\lambda_{\text{max}}$ of NQS is at 360 nm. Obviously LID has no absorption in the range of 300-700 nm. In order to eliminate the interference, the determination of product is fixed at 481 nm against the reagent blank.

3.2. Composition of product

The continuous variation method of equivalent mole method was used to determine the composition of Product I. The result is shown in Figure 2. As can be seen, the mole ratio of LID and NQS of Product I is 1:2.

3.3. Reaction mechanism

It has been reported that NQS could react with amino group of primary and secondary amine derivative (Quan-Min et al., 2007). Similarly, amino group of LID, taking on nucleophilicity due to the lone electron pair of nitrogen atoms, trends to attack on the electron-deficient center in NQS, namely no. 4 carbon atom (3,4 C-C can bond conjugate with 2-C O, as a result 4-C of NQS becomes electron lacking center). At the same time, it has been proved that the composition of Product I is 1:2 of LID and NQS. So it is concluded that two amino groups of lisinopril react with two NQS molecules. The reaction equation is shown in Scheme 2.

3.4. Optimization of reaction variables

3.4.1. Effect of pH

The influences of pH on the absorbance of product (I) is shown in Figure 3 at pH 4.0-7.0, the absorbance of the product is close to 0, indicating that under high acidity, LID has difficulty reacting with NQS. The possible reason may be that the amino group (—NH$_2$) of LID is protonated and turned into protonated amine salt (—NH$_3^+$). So it loses nucleophilic capability for 4-sodium sulfonate of NQS, and the nucleophilic substitution reaction can’t take place easily. If pH>7.0, the absorbance of the solution increases rapidly with the growth of pH. It may

![Absorption spectra of LID (10µg ml$^{-1}$) against water (1), NQS (0.3%, w/v) against water (2), and the reaction product of LID (10µgml$^{-1}$) with NQS against reagent blank (3).](image1)

![Determination of Product formation by continuous variation method. VR: NQS (2.5x10$^{-3}$ M); VD: lisinopril dihydrate (2.5x10$^{-3}$ M); VR + VD = 10 ml](image2)

![Scheme 1. Lisinopril dihydrate (LID)](image4)

![Scheme 2. Scheme for the reaction pathway of Lisinopril with NQS.](image5)
be that protonated amine salt (_NH3+) of LID turns into amino group (_NH2) again when the acidity of the solution becomes low. The higher the pH is, the more effectively the protonated amino group removes the proton, and the more easily the nucleophilic substitution reaction happens. At pH12.0, the absorbance reaches its maximum; in other words, the degree of the nucleophilic substitution reaction is also maximal. If pH>12.0, the absorbance of solution decreases sharply again. Presumably it may be that the increase of hydroxide ion holds back the nucleophilic substitution reaction between LID and the chromogenic reagent. Consequently, the absorbance of the solution reduces. In order to keep the high sensibility for determination of lisinopril dihydrate, pH 12.0 was selected for the optimal experimental conditions.

3.4.2. Effect of temperature

Keeping pH at 12.0, the influences of temperature on the absorbance of the solution were studied. It was found that the absorbance of solution was maximal at room temperature. Then it decreases rapidly with increasing temperature. In order to make the determination of LID both sensitive and simple, the room temperature was chosen as the optimum reaction condition. (Figure 4)

3.4.3. Effect of standing time

Keeping the experimental conditions unchanged, the absorbance of the solution of LID was measured after standing for various time periods Figure 5. The results show that LID reacts immediately with NQS at room temperature. 5 min is selected as the optimum condition.

3.4.4. Effect of concentration of NQS

According to the procedure, the absorbance of mixture solution of 10µg/mL was measured with different concentration and volume of NQS, the result was shown in Figures 6 and 7 respectively.

3.5. Validation of the Methods

3.5.1. Calibration and sensitivity

A linear correlation was found between absorbance at \( \lambda_{max} \) and concentration of LID. Beer’s law was obeyed over the concentration ranges given in Table 1, and the calibration graphs showed negligible intercept as described by the regression equation. \( Y = a + bX \) (where Y is the absorbance, a is the intercept, b the slope and X...
the concentration in µg/ml). The limits of detection (LOD) and quantification (LOQ) calculated according to the current ICH guidelines (ICH, 2005) are presented in Table 1. The other sensitivity parameters such as molar absorptivity and Sandell sensitivity are also contained in Table 1.

### 3.5.2. Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within the working limits) were analyzed, each determination being repeated three times. The relative standard deviations (%) were less than 2 and indicate the high accuracy and precision for the methods (Table 2). For intra-day and inter-day precision the relative standard deviation values were in the range of 0.49-1.86 % and represent the best appraisal of the methods in routine use.

### TABLE 1. Parameters for the performance of the proposed method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LID</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}}, \text{nm (Drug)} )</td>
<td>206</td>
</tr>
<tr>
<td>( \lambda_{\text{max}}, \text{nm(product)} )</td>
<td>481</td>
</tr>
<tr>
<td>Beer’s law limits, µg/ml</td>
<td>5-50</td>
</tr>
<tr>
<td>Molar absorptivity, l/mol cm</td>
<td>5.4 X 103</td>
</tr>
<tr>
<td>Sandell sensitivity, µg/cm²</td>
<td>0.0817</td>
</tr>
<tr>
<td>Limit of detection, µg/ml</td>
<td>1.16</td>
</tr>
<tr>
<td>Limit of quantification, µg/ml</td>
<td>3.53</td>
</tr>
<tr>
<td>Regression equation, Y*:</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0683</td>
</tr>
<tr>
<td>Standard deviation of intercept</td>
<td>0.00385</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0109</td>
</tr>
<tr>
<td>Standard deviation of slope</td>
<td>0.0004</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9997</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.00479</td>
</tr>
</tbody>
</table>

*Y=a+bX, where Y is the absorbance, a intercept, b slope and X concentration in µg/ml.

### TABLE 2. Evaluation of intra-day and inter-day accuracy and precision.

<table>
<thead>
<tr>
<th>LID Taken µg/mL</th>
<th>Intra-day(n=5)</th>
<th>Inter-day(n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found µg/mL</td>
<td>%RE</td>
<td>%RSD</td>
</tr>
<tr>
<td>40</td>
<td>40.12</td>
<td>1.24</td>
</tr>
<tr>
<td>50</td>
<td>49.84</td>
<td>0.78</td>
</tr>
<tr>
<td>60</td>
<td>60.16</td>
<td>0.49</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Recovery* (%)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard pH</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>12.2</td>
</tr>
<tr>
<td>NQS concentration (w/v %)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>

* Values are mean of three determinations.

### 3.5.3. Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed while the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were shown in Table 3.

### TABLE 3. Robustness of the proposed spectrophotometric method.

*Y=a+bX, where Y is the absorbance, a intercept, b slope and X concentration in µg/ml.

* Values are mean of three determinations.

### Figure 6. Effect of NQS concentrations on the reaction of LID with NQS. LID (100 µg/ml):1ml; NQS: 1ml; buffer solution (pH 12): 1.5ml; temperature: 25°C; reaction time: 5 min.

### Figure 7. Effect volume of NQS on absorbance of product LID with NQS. LID(100 µg/ml):1ml; NQS: 0.3%; buffer solution (pH 12): 1.5ml; temperature: 25°C; reaction time: 5 min.
3.5.4. Recovery Study

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of LID to reanalyzed solutions of commercial tablets Table 4.

3.6 Application of the Method

The proposed method was applied to some pharmaceutical formulations containing LID. The results in Table 4 indicate the high accuracy of the proposed method for the determination of the studied drug. As can be seen from Table 5, the proposed method has the advantage of being virtually free from interferences by excipients such as glucose, lactose and starch or from common degradation products. The percentage was 99.58± 0.80 for LID.

<table>
<thead>
<tr>
<th>TABLE 4. Recovery studies for determination of lisinopril by the proposed method.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample content (µg/ml)</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

* Values are mean of three determinations.

<table>
<thead>
<tr>
<th>TABLE 5. Determination of lisinopril formulations by the proposed method.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet brand **name</td>
</tr>
<tr>
<td>Lisopril</td>
</tr>
</tbody>
</table>

* Mean value of five determinations.
** Marketed by: Pharma International, Jordan.

Conclusion

The reported method is rapid, simple, economical, and fairly sensitive with regard to spectrophotometric methods. It can be used in routine analysis of pharmaceutical formulations of Lisinopril Dihydrate in quality control laboratories. Moreover, the present method can be directly applied to the pharmaceutical sample without prior separation or treatment.

References

[14] Gallo-Martinez L, Sevilla-Cabeza A, Camp’ins-Falc P, and Bosch-Reig F. 1998 A new derivatization procedure for the determination of cephalaxin with 1,2-naphthoquinone4-sulphonate in pharmaceutical and


