SPECTROPHOTOMETRIC METHOD FOR LISINOPRIL DETERMINATION USING NINHYDRIN

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Abstract

This paper presents a sensitive, accurate and rapid method for quantitative determination of lisinopril dihydrate (LIS) in bulk and pharmaceutical formulations based on the reaction with ninhydrin in the presence of potassium hydroxide. The reaction quantitatively proceeds at a temperature of 95 ± 2ºC, in 10 min and the end product, purple coloured, exhibits maximum absorption at 567 nm. The Lambert-Beer law is obeyed within the range of 10 – 30 µg/mL, while the calibration curve is described by the regression equation \[ A = 0.0312 C \text{ (µg/mL)} – 0.2262 \], having a correlation coefficient of \( R^2 = 0.9981 \) (n = 8). The influence of temperature, heating time and reactive concentrations on the end product was also analyzed, so as to render the optimal conditions for reaction. In order to validate the method, the linearity, the limit of detection (LOD), limit of quantification (LOQ), sensitivity, selectivity, precision, accuracy and recovery were determined according to ICH guidelines. The LOD and LOQ values were calculated to be 1.16 µg/mL and 3.52 µg/mL respectively. Intra-day and inter-day accuracy expressed as relative error was less than 1.12% with precision quantified as relative standard deviation ranging from 1.14 to 1.98%. The percent recovery ranged from 98.12 to 102.57%. No interferences from the common excipients were detected and therefore the method was considered specific. The proposed method was successfully applied for the quantitative determination of lisinopril from three pharmaceutical products, namely Lisigamma®, Lisinopril® Sandoz and Ranolip®.

Rezumat

Lucrarea prezintă o metodă spectrofotometrică sensibilă, precisă și rapidă de determinare cantitativă a lisinoprilului dihidrat (LIS) în substanța pură și din forma farmaceutică pe baza reacției cu ninhidrina în prezența hidroxidului de potasiu. Reacția decurge cantitativ la temperatura de 95 ± 2ºC în 10 min, iar produsul obținut, colorat violet, prezintă maxim de absorbție la 567 nm. Legea Lambert-Beer se respectă în intervalul de concentrații 10 – 30 µg/mL, iar dreapta de calibrare este descrisă de ecuația de regresie \[ A = 0.0312C \text{ (µg/mL)} – 0.2262 \], cu coeficientul de corelare \( R^2 = 0.9981 \) (n = 8). S-a studiat
înfluența temperaturii, a timpului de încălzire și a concentrațiilor reactivilor asupra produsului final și s-au stabilit condițiile opțime de reacție. În vederea validării metodei, s-au evaluat conform ghidurilor ICH linearitatea metodei, limita de detecție (LOD) și limita de cuantificare (LOQ), sensibilitatea, selectivitatea, precizia, acuratețea și procentul de regăsire ale metodei. Valori calculate corespunzătoare pentru LOD și LOQ sunt 1,16 µg/mL și respectiv 3,52 µg/mL. Acuratețea metodei, exprimată ca valoare a erorii relative, a fost mai mică de 1,12% cu o precizie exprimată ca valoare a deviației standard relative varind între 1,14 și 1,98%. Procentul regăsirii prin metoda propusă s-a situat între 98,12 și 102,57%. Nu s-au raportat interferențe cu excipientii utilizați în mod frecvent, fapt ce conduce la concluzia că metoda este specifică. Metoda propusă a fost aplicată cu succes la determinarea cantitativă a lisinoprilului din trei specialități farmaceutice: Lisigamma®, Lisinopril® Sandoz și Ranolip® comprimate.

**Keywords:** lisinopril, ninhydrin, spectrophotometry, pharmaceutical formulation

**Introduction**

Lisinopril dihydrate (Figure 1), \((2S)-1\text{-}[2S\text{-}6\text{-amino}-2\text{-}[2S\text{-}1\text{-hydroxy}-1\text{-oxo}-4\text{-phenylbutan}-2\text{-yl}]\text{hexanoyl}]\text{pyrrolidine}-2\text{-carboxilic acid dihydrate}\) (LIS) is an angiotensin converting enzyme (ACE) inhibitor, widely used for the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and post myocardial infarction [5,14]. The official methods for LIS determination in both bulk and tablets are potentiometric acid-base titration [44], and HPLC using octylsilyl silica gel column at 50ºC with phosphate solution-acetonitrile (96:4, v/v) as mobile phase [46].

![Figure 1](image-url)

**Figure 1**

Lisinopril dihydrate chemical structure

Analytical methods such as HPLC [4,9,18,24,35,40], LC/MS [17,26,29,35], gas chromatography with mass detection [20,21], densitometric HPTLC [10], polarography [1,8,33], capillary electrophoresis [15,16], spectrofluorimetry [9,11,13,43], radioimmunoassay [41], fluoroimmunoassay [42] have been developed for the determination of lisinopril in biological fluids and pharmaceutical formulations, either alone
or in combination with hydrochlorothiazide. Most of these methods require the use of expensive equipment and reagents. Spectrophotometric methods still have practical and economical advantages over the other methods, and thus are widely used for the assay of pharmaceuticals in bulk and dosage forms.

In the available literature several different spectrophotometric methods have been reported for LIS quantification in pharmaceutical dosage forms: using different reagents [3,6,7,9,19,27,30,32,34,37,43] and derivative UV spectrophotometric methods [10,12,25,28,39].

Among the reagents used for the assay of LIS in pharmaceutical formulations, ninhydrin has been employed in a few papers. Thus, the method reported by Rahman et al. [32] used ninhydrin in N,N’-dimethylformamide (DMF) medium at room temperature for the determination of LIS by initial-rate, rate-constant and fixed time (10 min) procedures, the last one having a molar absorptivity value of $4.70 \times 10^3 \text{Lmol}^{-1}\text{cm}^{-1}$. The reagent in DMF medium but involving elevated temperature ($80 \pm 5 ^\circ\text{C}$), when a greenish blue product which absorbs maximally at 600 nm was formed, have been used by Raza et al. [34] for the assay of LIS; the molar absorptivity value was $4.083 \times 10^3 \text{Lmol}^{-1}\text{cm}^{-1}$. The common feature of these two methods [32,34] is the use of DMF medium, an organic solvent which does not lack toxicity. The method reported by Basavaiah et al. [3] is based on the reaction of LIS with ninhydrin in aqueous medium in the presence of bicarbonate producing a yellow color product which exhibits maximum absorption at 420 nm; the molar absorptivity value was $2.02 \times 10^3 \text{Lmol}^{-1}\text{cm}^{-1}$.

This paper presents a sensitive and precise spectrophotometric method for the assay of LIS in bulk and pharmaceutical formulation based on the reaction of LIS with ninhydrin in ethanol medium in the presence of potassium hydroxide. The calculated molar absorptivity value is $7.98 \times 10^3 \text{Lmol}^{-1}\text{cm}^{-1}$. From the ninhydrin using methods, the proposed method has the greatest molar absorptivity value, and therefore the highest sensitivity. The method was also validated.

**Materials and Methods**

**Apparatus**

The spectrophotometric measurements were performed using a Spectronic Unicam - UV 300 UV-Visible Spectrometer with 1 cm matched glass cells.
Materials and reagents

All the chemicals used were of analytical reagent grade.

0.5% Ninhydrin. 500 mg of chemical (Merck) were dissolved in ethanol and brought to 100 mL with ethanol.

0.1 M Potassium hydroxide. The solution was prepared by dissolving 561 mg of chemical in ethanol and diluting to 100 mL in a calibrated flask.

Standard drug solution. The lisinopril dihydrate was kindly provided by Medochemie, Limassol (Cyprus). A stock standard solution of 1000 µg/mL LIS was prepared by dissolving 100 mg of pure drug in distilled water and diluting to 100 mL in a calibrated flask with water. The standard LIS solution (500 µg/mL) was prepared from the stock solution, by appropriate water dilution.

Proposed procedure

Different aliquots of 500 µg/mL LIS solution (10-30 µg/mL) were accurately measured and transferred in heating tubes. 1 mL of 0.5% ninhydrin and 0.1 mL of 0.1 M potassium hydroxide were added to each tube. The mixture was kept in a water bath at 95 ± 2 ºC for 10 minutes, then cooled to room temperature and transferred into a 25 mL volumetric flask. The volume was made up to the mark by adding water. The absorbance was measured at 567 nm against the reagent blank, which was similarly prepared by omitting the drug. The calibration curve was performed by plotting the measured absorbance values versus concentration.

Procedure for pharmaceutical formulation

Twenty tablets were accurately weighed and powdered. A quantity of powder containing 50 mg of lisinopril was transferred into a 100 mL volumetric flask with 60 mL water. The mixture was shaken for 15 min, diluted to volume with water and then filtered using 0.2 µm Nylon filter membrane. The filtrate was subsequently subjected to analysis using the above described procedure.

Results and Discussion

Ninhydrin reagent is used for the determination of aliphatic primary amines, amino acids, peptides and their related compounds [2,22,23,31,38].

LIS interacts with ninhydrin in the presence of potassium hydroxide in ethanol medium via oxidative deamination of the primary aliphatic amino group of the lisyne rest contained in the lisinopril molecule followed by the
condensation of the reduced ninhydrin to form the purple colored reaction complex with \( \lambda_{\text{max}} \) at 567 nm (Figure 2 and Scheme I).

![Figure 2](image_url)

The absorption spectra for the purple end product and blank

![Scheme I](image_url)

Suggested reaction pathway between LIS and ninhydrin

**Optimization of reaction conditions**

Different parameters such as the temperature, heating time, reagents concentration have been analyzed, in order to render the optimal conditions for reaction. It has been noted that the complete color development was attained at 95 ± 2 ºC. Optimum reaction time has been determined by heating the reaction mixture on a water bath at 95 ± 2 ºC. A heating time of 10 minutes was found as optimal for the development of the purple color product (Figure 3).
In order to investigate the effect of ninhydrin concentration on the reaction product color, the change in absorbance generated by varying the concentration of ninhydrin on fixed concentration of LIS (20 µg/mL) has been measured against reagent blank. The optimum value was found to be 1 mL of 0.5% ninhydrin (Figure 4).

![Figure 3. The effect of heating time over the color intensity](image)

![Figure 4. The effect of ninhydrin over the color intensity](image)

It has been observed that a volume over 0.1 mL of 0.1 M potassium hydroxide produces a yellow-brownish reaction product color. Therefore, 0.1 mL of 0.1 M potassium hydroxide was found as being optimum for the development of the purple reaction product. All measurements were performed at 567 nm, against the blank.

**Method validation**

The proposed method has been validated according to the present ICH guidelines [45].

**Linearity and sensitivity**

Under optimum conditions, a linear correlation between the absorbance and the concentration of LIS was obtained in the range of 10-30 µg/mL LIS. The calibration curve is described by the equation:

\[ Y = a + bC \]

where \( Y \) = absorbance, \( a \) = intercept, \( b \) = slope and \( C \) = concentration in µg/mL, which were obtained by the least squares method. The intercept, slope and correlation coefficient are presented in Table I. The sensitivity parameters such as molar absorptivity and Sandell sensitivity are also presented in Table I.

**Limits of detection and quantification**

The ICH guidelines were followed in order to determine the LOD and LOQ. Accordingly, the method based on the standard deviation of the response and the slope has been applied, so that 3.3 and 10 times the standard deviation values of y-intercept of regression line and the regression
equation were used to calculate the LOD and LOQ. The computed values are presented in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) / nm</td>
<td>567</td>
</tr>
<tr>
<td>Linariety range, ( \mu \text{g/mL} )</td>
<td>10-30</td>
</tr>
<tr>
<td>Molar absorptivity (( \varepsilon )) ( \text{L/(mol}\cdot\text{cm}) )</td>
<td>(7.98 \times 10^3)</td>
</tr>
<tr>
<td>Sandell sensitivity, ( \mu \text{g/cm}^2/0.001 \text{ abs unit} )</td>
<td>0.0640</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0312</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.2262</td>
</tr>
<tr>
<td>Limit of detection (LOD) ( \mu \text{g/mL} )</td>
<td>1.16</td>
</tr>
<tr>
<td>Limit of quantification (LOQ) ( \mu \text{g/mL} )</td>
<td>3.52</td>
</tr>
<tr>
<td>Standard deviation of slope (( S_b ))</td>
<td>±0.0011</td>
</tr>
<tr>
<td>Standard deviation of intercept (( S_a ))</td>
<td>± 0.0111</td>
</tr>
<tr>
<td>Correlation coefficient (( r ))</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

**Selectivity**

The proposed method was tested in order to assess its selectivity using the artificial mixture analysis. It has been confirmed that the measured absorbance was only produced by the analyte. A synthetic mixture was prepared, containing lisinopril (50 mg) as lisinopril dihydrate (54.44 mg), talc (100 mg), starch (150 mg), lactose (180 mg), magnesium stearate (50 mg) and calcium diphosphate (75 mg). The extract was yielded according to the procedure that was described for tablets and subsequently analyzed using the procedure previously described. The replicate analysis (\( n = 5 \)) for a concentration level of 20 \( \mu \text{g/mL} \) LIS has yielded the \% LIS recovery at 100.70 ± 1.79, and thus revealed that the inactive ingredients did not interfere with LIS determination.

**Precision and accuracy**

Intra-day and inter-day precision values have been calculated by replicate analysis (\( n = 5 \)) of calibration standard, at three different concentration levels, during the same day, and then during five consecutive days. The RSD (\%) values of intra-day and inter-day measurements have indicated a good precision. (Table II)
Accuracy, defined as the closeness between the reference and the found values, has been evaluated, on the other hand, as percentage relative error between the measured and theoretical concentration of LIS. The results are presented in Table II, and show good accuracy for this method.

### Table II

<table>
<thead>
<tr>
<th>LIS taken, µg/mL</th>
<th>LIS found, µg/mL</th>
<th>Intra-day accuracy and precision</th>
<th>Inter-day accuracy and precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RE, %</td>
<td>RSD, %</td>
</tr>
<tr>
<td>10</td>
<td>9.94</td>
<td>-0.60</td>
<td>1.14</td>
</tr>
<tr>
<td>20</td>
<td>19.87</td>
<td>-0.65</td>
<td>1.56</td>
</tr>
<tr>
<td>25</td>
<td>24.72</td>
<td>-1.12</td>
<td>1.98</td>
</tr>
</tbody>
</table>

RE - Relative error; RSD - Relative standard deviation.

### Application to pharmaceutical formulation

The proposed method was applied for the quantification of LIS, in tablets pertaining to three commercial formulations. The results were compared to those obtained with the reference method [3], using the Student’s t-test for accuracy and F-test for precision. The results as presented in Table III reveal no significant differences between the proposed method and the reference method. The Student’s t- and the F-values at 95 % confidence level are less than the theoretical one, but nevertheless confirming a good agreement between the results obtained by the proposed method and the reference method.

### Table III

<table>
<thead>
<tr>
<th>Tablet brand name</th>
<th>Label claim, mg/tablet</th>
<th>Found(^a) ( label claim ± SD), %</th>
<th>Reference method</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lisigamma(^bc)</td>
<td>10</td>
<td>101.152 ± 0.853</td>
<td>102.153 ± 1.689</td>
<td>t = 1.088</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F = 3.921</td>
</tr>
<tr>
<td>Lisinopril Sandoz(^bc)</td>
<td>10</td>
<td>100.367 ± 0.641</td>
<td>100.668 ± 1.362</td>
<td>t = 0.557</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F = 4.510</td>
</tr>
<tr>
<td>Ranolip(^bd)</td>
<td>10</td>
<td>100.456 ± 0.691</td>
<td>101.118 ± 0.898</td>
<td>t = 1.966</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F = 1.690</td>
</tr>
</tbody>
</table>

\(^a\) Mean value of five determinations; \(^b\) Worwag Pharma GmbH & Co, Germany; \(^c\) Salutas Pharma GmbH, Germany; \(^d\) Terapia-Ranbaxy, Romania

The tabulated F value at 95% confidence level, for four degrees of freedom, is 6.39

The tabulated t value at 95% confidence level, for four degrees of freedom, is 2.77
Recovery analysis

The validity of the proposed method was further demonstrated by performing recovery studies. Pre-analyzed tablet powder was spiked with pure LIS at three concentration levels (50, 100 and 150 % of that present in tablet powder), and the total has been found using the proposed method. The percentage values for LIS added recovery are ranging between 98.12 and 102.57, with a standard deviation of 0.40 – 1.94%, which reveals a good recovery.

Table IV

<table>
<thead>
<tr>
<th>Tested tablet</th>
<th>LIS in tablet, µg/mL</th>
<th>Pure LIS added, µg/mL</th>
<th>Total found, µg/mL</th>
<th>Pure LIS recovered ± SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lisigamma</td>
<td>10.31</td>
<td>5</td>
<td>15.16</td>
<td>98.20 ± 0.93</td>
</tr>
<tr>
<td></td>
<td>10.31</td>
<td>10</td>
<td>20.31</td>
<td>100.01 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>10.31</td>
<td>15</td>
<td>25.27</td>
<td>99.74 ± 1.47</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>10.07</td>
<td>5</td>
<td>15.27</td>
<td>102.57 ± 1.34</td>
</tr>
<tr>
<td>Sandoz</td>
<td>10.07</td>
<td>10</td>
<td>20.13</td>
<td>100.58 ± 1.94</td>
</tr>
<tr>
<td></td>
<td>10.07</td>
<td>15</td>
<td>24.47</td>
<td>98.12 ± 1.37</td>
</tr>
</tbody>
</table>

* Mean value of three measurements

Conclusions

This paper presents a new spectrophotometric method that has been developed and validated for the quantification of lisinopril dihydrate from its pharmaceutical formulations. The proposed method was proven to be selective, sensitive, rapid and inexpensive.

References

44. ***, European Pharmacopoea, 5th Ed., Council of Europe, Strasbourg, 2004, p.1922-1923

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