METHOD VALIDATION FOR SPECTROPHOTOMETRIC DETERMINATION OF LISINOPRIL IN PHARMACEUTICALS USING COPPER SULPHATE

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Abstract

This paper presents the statistical validation for the spectrophotometric method of lisinopril determination in bulk and pharmaceutical formulations. The method presented herein underpinned by the reaction between lisinopril and copper sulphate. The maximal absorption for the blue complexation reaction product occurs at a 740 nm wavelength. Accordingly, the calibration curve is described by a second order polynomial regression equation, within the range of 0.1 to 0.7 mg/ml: $A = -0.3171C^2 + 0.9653C + 0.0028$, where $A$ represents the measured absorbance at $\lambda = 740$ nm using cells of 5 cm thickness, and $C$ stands for the concentration expressed in mg/ml, hence rendering $R^2 = 0.9999$ as correlation coefficient. The limits of detection (LOD) and quantification (LOQ), calculated according to ICH guidelines, are 0.026 and 0.079 mg/ml, respectively. Intra-day and inter-day accuracy expressed as relative error are better than 2.5%, while the precision quantified by the relative standard deviation is less than 3.2%. The method was successfully applied for the analysis of two tablet brands containing lisinopril: Lisigamma and Lisinopril Antibiotice Iaşi 10 mg.

Keywords: lisinopril, copper sulphate, spectrophotometry, pharmaceutical formulation.
INTRODUCTION

Lisinopril dihydrate (LIS, Figure 1), (2S)-1-[(2S)-6-amino-2[(2S)-1-hydroxy-1-oxo-4-phenylbutan-2-yl]amino]hexanoyl]pyrrolidine-2-carboxilic acid dihydrate is an angiotensin converting enzyme (ACE) inhibitor, the lysine analog of enalaprilat, which is the active metabolite of enalapril. It is used for the treatment of essential hypertension, congestive heart failure, diabetic nephropathy and post-myocardial infarction [1,2]. The official methods for the determination of LIS in pure and tablet forms are potentiometric acid-base titration [3] and HPLC using octylsilyl silica gel column at 50 °C, along with phosphate solution-acetonitrile (96:4, v/v) as mobile phase [4].

![Chemical Structure](attachment:image.png)

**Figure 1.** Lisinopril dihydrate: chemical structure.

Several analytical methods have been described for the determination of lisinopril in biological fluids or pharmaceutical formulation, whether alone or in combination with hydrochlorothiazide: HPLC [5-10], gas chromatography with mass detection [11, 12], LC/MS [13-15], densitometric HPTLC [16], capillary electrophoresis [17, 18], spectrofluorimetry [6, 19, 20], polarography [21-23]. Most of the above mentioned techniques are sensitive but cumbersome and expensive. Within this paper, the spectrophotometric methods represent the technique of choice due to their inherent simplicity and economical advantages; nevertheless, they are already used for the assay of a wide variety of pharmaceuticals, in bulk and dosage form. Moreover, several literature references are reporting spectrophotometric methods as valid tools for the assay of LIS in pharmaceutical formulations, which are based either on the reaction with different reagents [6,24-30] or on derivative UV-spectrophotometry [31-33]. Most of these methods involve organic solvents as reaction medium (often undesirable because of their toxicity), require longer heating time, and use expensive reagents.

This paper tries to deal with the above stated drawbacks, by presenting a simple, fast, economical and environmentally-friendly spectrophotometric method, for the assay of lisinopril in pure and pharmaceutical formulations. The method is based on the reaction between lisinopril and copper sulphate, in water medium, at room temperature. No organic solvent and no heating time were required.

MATERIALS AND METHODS

**Apparatus**

All spectrophotometric measurements were performed using a SPECTRONIC UNICAM – UV 300 UV-VISIBLE SPECTROMETER, with 5 cm matched glass cells.

**Materials and reagents**

All chemicals used within this paper’s experiments are of analytical reagent grade.

10 mg/ml Copper sulphate 5-hydrate. 2000 µg of chemical (Reactivul Bucureşti) was dissolved in distilled water and made up to 200 ml with the solvent.

**Standard drug solution.** Lisinopril dihydrate was kindly provided by Medochemie, Limassol, Cyprus and it was used as received. A standard stock solution of 5 mg/ml LIS was prepared by dissolving 500 mg pure drug in distilled water and then diluting it to 100 ml in a calibrated flask with water. The standard LIS solution (2.5 mg/ml) was prepared from the stock solution, by appropriate water dilution.

**Proposed procedure**

Aliquots of 2.5 mg/ml LIS solution (0.1–0.7 mg/ml) were accurately measured and transferred into a series of 25 ml standard volumetric flasks. 10 ml of 10 mg/ml copper sulphate 5-hydrate were added to each flask. The volume was made up to the mark with distilled water. The absorbance was measured using the 5 cm cells at 740 nm, against reagent blank (i.e. similarly prepared, but omitting the drug). The calibration graph was generated by plotting the measured absorbance values, according to concentration variation.

**Pharmaceutical formulation**

Twenty tablets were accurately weighed and powdered. A quantity of powder, containing 100 mg of lisinopril, was transferred into a 50 ml volumetric flask with 30 ml water. The mixture was shaken for 15 min, diluted to volume with water, and then filtered. The filtrate was subjected to analysis, using the above described procedure.

RESULTS AND DISCUSSION

As presented in [34], the reaction between LIS and copper sulphate is a reversible reaction. For shifting this equilibrium towards the final product, an excess of copper ions is required. For the given reaction conditions, the rank of the matrix of the absorbance values proves that only one final product is to be obtained [34]. The complexation product has a maximum absorbance at 740 nm (see Figure 2).
The effect of the CuSO₄·5-hydrate concentration

In order to study the effect of CuSO₄·5-hydrate concentration on the reaction product color, varying volumes of 10 mg/ml CuSO₄·5-hydrate (2.5–15 ml) were reacted with 6 ml of 2.5 mg/ml LIS into a 25 ml volumetric flask. The absorbance has been measured against reagent blank. 10 ml of 10 mg/ml CuSO₄·5-hydrate has been found as the acceptable tradeoff value (Figure 3).

Method validation

Linearity

The ICH guidelines mention that, for some analytical procedures which do not demonstrate linearity, the analytical response should be described by an appropriate function of concentration for an analyte within a sample [35].

The calibration graph is described by the second order polynomial regression equation:

\[ A = a + b_1 C + b_2 C^2 \]

where \( A \) is the absorbance and \( C \) is the concentration in mg/ml (Figure 4). Optical characteristics and statistical data, for the regression equation of the proposed method are presented in Table 1.

Limit 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 has been applied, so that three and ten times the standard deviation values for the blank and regression equation were used to calculate the LOD and LOQ. The computed values were found to be 0.026 and 0.079 mg/ml, respectively.

Selectivity test

The proposed method was tested for selectivity by artificial mixture analysis. An artificial mixture containing lisinopril (20 mg) as lisinopril dihydrate (21.78 mg), starch (50 mg), magnesium stearate (20 mg) and calcium diphosphate (10 mg) was prepared. The extract was obtained according to the procedure described for tablets and analyzed using the earlier described procedure. The replicate analysis (\( n = 5 \)) of the 0.3 mg/ml LIS concentration level yielded the % recovery of LIS at 99.74 ± 1.32, therefore revealing that the inactive ingredients did not interfere with LIS determination.

It can be concluded that our selectivity test has confirmed that the measured absorbance was produced only by the analyte. The study also pointed out that the inactive ingredients, such as yellow iron oxide, red iron oxide and talc do interfere with the active substance, and hence the method cannot be applied for lisinopril quantification in the presence of the above mentioned ingredients.
**Precision and accuracy**

The precision and accuracy of the method have been evaluated 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 studies have revealed that good precision was achieved (Table 2). Accuracy has been calculated as relative error (%) between the found concentration and its theoretical counterpart (see Table 2).

**Application to pharmaceutical formulation**

The proposed method was used for the quantification of LIS in two tablet commercial formulations. The results were compared with those obtained with the reference method [35], by using Student’s t-test for accuracy and F-test for precision. The results (presented in Table 3) have failed to reveal any significant difference between the proposed method and the reference method. The values obtained with student’s t-test and F-test at a 95% confidence level are smaller than the theoretical ones, thereby confirming a good agreement with the reference method.

**Recovery study**

The validity of the proposed method was further demonstrated using recovery studies. Pre-analyzed tablet powder was spiked with pure LIS at three concentration levels (50, 100, and 150% of that from the tablet powder) and the total has been found by applying the proposed method. The percentage values of LIS added recovery ranged between 97.63 and 101.22, with a standard deviation of 0.92 – 2.57%; all these results reveal a good degree of recovery (see Table 4).

**CONCLUSIONS**

The novel spectrophotometric method presented in this paper, which was developed and validated for the quantification of LIS in pharmaceutical formulations is a simple, fast and inexpensive method that involves only one reagent. It has the all the advantages of the fact that requires only simple operations, including the possibility of carrying them out with the common laboratory instruments. Moreover, this method is also environment-friendly since it excludes the use of organic solvents. Further research will focus on a thorough analysis, regarding the advantages and drawbacks of this method in comparison with the state-of-the-art.

### Table II. Evaluation of intra-day and inter-day accuracy and precision.

<table>
<thead>
<tr>
<th>LIS taken, mg/ml</th>
<th>Intra-day accuracy and precision</th>
<th>Inter-day accuracy and precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIS found, mg/ml</td>
<td>RE, %</td>
</tr>
<tr>
<td>0.2</td>
<td>0.1988</td>
<td>-0.59</td>
</tr>
<tr>
<td>0.4</td>
<td>0.3956</td>
<td>-1.11</td>
</tr>
<tr>
<td>0.6</td>
<td>0.5972</td>
<td>-0.47</td>
</tr>
</tbody>
</table>

RE: Relative error; RSD: Relative standard deviation.

### Table III. Determination of lisinopril formulation by the proposed and reference method.

<table>
<thead>
<tr>
<th>Tablet brand name</th>
<th>Label claim, mg/tablet</th>
<th>Found(^a) (label claim ± SD), %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reference method</td>
</tr>
<tr>
<td>Lisigamma(^b)</td>
<td>10</td>
<td>102.35 ± 0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( t = 1.63)</td>
</tr>
<tr>
<td>Lisinopril Antibiotice(^c)</td>
<td>10</td>
<td>105.68 ± 0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( t = 2.36)</td>
</tr>
</tbody>
</table>

\(^a\) Mean value of five determinations; \(^b\) Worwag Pharma GmbH & Co, Germany; \(^c\) Antibiotice Iași, 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.

### Table IV. Results of recovery study by standard-addition method.

<table>
<thead>
<tr>
<th>Tablet studied</th>
<th>LIS in tablet, mg/ml</th>
<th>Pure LIS added, mg/ml</th>
<th>Total found, mg/ml</th>
<th>Pure LIS recovered(^d) ± SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lisigamma</td>
<td>0.207</td>
<td>0.1</td>
<td>0.307</td>
<td>100.87 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>0.207</td>
<td>0.2</td>
<td>0.404</td>
<td>98.91 ± 2.57</td>
</tr>
<tr>
<td></td>
<td>0.207</td>
<td>0.3</td>
<td>0.510</td>
<td>101.22 ± 2.44</td>
</tr>
<tr>
<td>Lisinopril Antibiotice</td>
<td>0.211</td>
<td>0.1</td>
<td>0.308</td>
<td>97.63 ± 1.38</td>
</tr>
<tr>
<td></td>
<td>0.211</td>
<td>0.2</td>
<td>0.412</td>
<td>100.70 ± 2.05</td>
</tr>
<tr>
<td></td>
<td>0.211</td>
<td>0.3</td>
<td>0.512</td>
<td>100.55 ± 0.92</td>
</tr>
</tbody>
</table>

\(^d\) Mean value of three measurements.
References