Determination of Lisinopril in Pure and Tablet form by Using 2-Hydroxynaphthaldehyde as Derivatizing Reagent

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Abstract
An easy, sensitive and accurate spectrophotometric method has been developed for the determination of Lisinopril (LNP) in pure and tablet formulations based on derivatization reaction with 2-hydroxynaphthaldehyde (2HNA). The derivatization reaction was carried out in methanol solvent at pH-5.5 at 95±2°C for 15 min. The linear calibration curve was obtained that obeyed the Beer’s law within the concentration range 5-50 µgmL⁻¹ of LNP at 433 nm with a coefficient of determination R²=0.996. The recovery was in the range from 98.25-101.82 with molar absorptivity of drug 9×10³ mole⁻¹cm⁻¹. The method was accurate and precise (intra-day variation 0.05-0.97% and inter-day 0.07-1.6%), with limit of detection (LOD) and limit of quantification (LOQ) 0.264 and 0.8 µgmL⁻¹, respectively. No interferences from the excipients were detected. The method was applied for the rapid analysis of LNP in pharmaceutical products.

Keywords: Lisinopril, Spectrophotometry, 2-hydroxynaphthaldehyde, Derivatization

Introduction
Lisinopril 1-[6-Amino-2-(1-carboxy-3-phenyl-propylamino)-pyrrolidine-2-carboxylic acid. It has a molecular formula of C₂₁H₃₁N₃O₅ and a molecular weight of 441.52 gmol⁻¹ [1-3]. The structure of drug LNP & reagent 2HNA are given below (Fig.1).

LNP, is the third ACE inhibitor permitted for use in the United States; LNP itself is active, unlike enalaprilat (ENA). LNP is a significantly more potent inhibitor of ACE than enalaprilat in vitro. Both are used for heart failure and hypertension treatment and diuretic medications. LNP is an angiotensin converting enzyme inhibitor used in the medication of hypertension and heart failure in prophylactic treatment following myocardial infarction and diabetic nephropathy. LNP are amongst the key therapeutic developments of modern medicine due to their histronic impact in the treatment of congestive cardiac failure and arterial hypertension. The renin-angiotensin system is instantaneously stimulated as a reflex response in order to conserve blood volume.
The LNP causes blood pressure reduction by 5-6 mm Hg, due to which 40% hazard of stroke and 15-20% coronary heart disease can be decreased. Various classes of allopathic drugs such as diuretics, antagonists of adrenergic receptors, adrenergic agonists, blockers of calcium channels, ACE inhibitors, antagonists of angiotensin II receptors, antagonists of aldosterone, vasodilators and centrally acting adrenergic drugs are used in the maintenance and treatment of all types of hypertension [4].

It has been observed that in some cases, sub-standard medicinal drugs which do not contain the amount of active ingredient stated on the label are sold in the market. This has prompted the quality control labs to do random sampling of the marketed drugs and determine the active ingredient content for quality control purpose. In developing countries, the quality control labs do not have expensive, sophisticated equipment to embark upon the drug quality control task.

The literature reveals various analytical methods were described for the determination of LNP such as titrimetric [5, 6], Spectrophotometric [7-15], spectrofluorometric [16-20], chromatographic [21-38], derivative UV-spectrophotometric, [39-40] polarography [41-42], radioimmunoassay [43] and fluoroimmunoassay [44]. Spectroscopy is still the most widely used analytical tool for major qualitative and quantitative analysis of pharmaceutical formulations. It delivers key financial and experimental advantages over other techniques. For example, many derivative spectrophotometric methods were established using different reagents, one of them Paraskevas and co-workers developed a spectrophotometric method [12] in single and multi-component tablets also containing hydrochlorothiazide (HCT), based on the derivatization reaction with 1-fluoro-2,4-dinitrobenzene (FDNB, Sanger reagent). The active ingredient contents of drug in pure and dosage form were determined, using acetonitrile solvent, at pH 8.2 (borate buffer) in the dark at 60 °C for 45 min. The LNP complex was measured at $\lambda_{max}$ 356.5 or 405.5 nm (only at 405.5 nm if HCT is present).

Another method was proposed by Shärlcea [45] and her team for the quantitative determination of LNP 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 ± 2°C, in 10 min and the end product, purple colored, exhibits maximum absorption at 567 nm.
Finally, the latest method was developed by Shraitah [46] based on the reaction of Alizarin with primary amine present in the LNP in the presence of 80% ethyl alcohol. The reaction produced a complex red colored product that absorbs maximally at 434 nm. The above reported methods were not only time consuming but also involve expensive solvents, and thus hard to apply in routine analysis. This prompted us to develop a simple, economical and rapid spectrophotometric procedure to determine LNP from pure and drug formulations. The process is based on Schiff base reaction by an aldehyde or ketones reacting with a primary amino group under acid or base catalysis or heat. In the same way, amino group of LNP reacts with the aldehyde group of 2HNA reagent. The following general reaction is shown in scheme 1.

Scheme 1. Principle of reaction (schiff’s base reaction)

Materials and Methods
Reagents and Chemicals

All of the materials and reagents used were of analytical grade. LNP (100.21%) was obtained from Bosch Pharmaceutical (Pvt.) Ltd. The reagent 2HNA (100.1%) was purchased from EMD Chemicals (Gibbstown, NJ, USA), acetic acid, potassium chloride, hydrochloric acid, sodium carbonate, sodium bicarbonate, methanol, ethanol were from Merck, Germany. Sodium acetate was from Fluka, Switzerland. De-ionized water was used throughout the study.

Commercial Tablets

Tablets CORACE (Bosch Pharmaceutical Pvt. Ltd.), ZESTRIL (ICI Pakistan Ltd.), TRUPRIL (Getz Pharma Pakistan), which obtained labeled amount of 10 mg/tablet, while LISNA (ZAFAR Pharmaceutical Lab. Pakistan) contained 20 mg/tablet LNP and purchased from local market Hyderabad, Sindh, Pakistan.

Stock Solutions
Standard solutions

The stock solution (0.02% w/v) of drug LNP was prepared by dissolving exact weighed 20 mg in 10 mL volumetric flask in methanol. Then 1mL above solution diluted up to 10 mL calibrated volumetric flask in the same solvent. The stock solution (1% w/v) of reagent 2HNA was freshly prepared by dissolving 0.1 gm in sufficient methanol and the volume was adjusted to 10 mL, achieving 10 mgmL\(^{-1}\) concentrations.

Buffer solutions

The buffer solutions were prepared of pH 1-2 by utilizing (0.1M) hydrochloric acid and (0.1M) potassium chloride, pH 3-5.7
(0.1M) acetic acid and (0.1M) sodium acetate, pH 5.8-8 sodium phosphate monobasic and sodium phosphate dibasic, pH 8-10 sodium carbonate and sodium bicarbonate.

**Instrumentations**

All measurements were performed using the Perkin Elmer double beam spectrophotometer, Lambda 35 UV/Visible spectrometer (USA), connected to Dell computer, using 1 cm quartz cuvette, and the pH meter was used by model Orion 420A pH meter fitted with a glass electrode and reference electrode (Orion Research Inc. Boston, USA).

**Method-1**

*Analysis of standard drug LNP*

The LNP drug solutions 1-5 mL were dissolved in methanol at concentrations 5-50 µgmL⁻¹, and 1 mL of each drug solution was transferred to 10 mL calibrated volumetric flask, followed by the addition of 2 mL of reagent 2HNA (1% w/v) prepared in methanol and 1 mL alcoholic acetate buffer pH-5.5. The mixture was heated for 15 min at 95 °C ± 1°C on a water bath. Then flask contents were allowed at room temperature and the volume was adjusted with methanol up to the mark. The absorbance was finally measured at 433 nm against the blank.

**Method-2**

*Application on analysis of commercial tablets*

An accurately weighed mass of 20 tablets was crushed. The powder of tablets equivalent to 20 mg LNP was weighed and transferred into a 100 mL volumetric flask containing a sufficient amount of solvent methanol. The suspension was stirred for 10 min. The solution was filtered through a filter paper (Whatman No. 1), after rinsing with methanol the volume was adjusted to 100 mL. Then 1 mL of the resulting solution was shifted to 10 mL stoppered volumetric flask, and 2 mL of reagent 2HNA was added, followed by 1 mL acetate buffer pH-5.5. The contents were heated up to 15 min at 95°C ± 1°C on a water bath, cooled at room temperature (25°C), the volume was adjusted and absorbance recorded at 433 nm, against the blank.

**Validation of method for derivatization**

A newly developed spectrophotometric method for the determination of imine derivative of LNP was validated for linearity, accuracy, % recovery, sensitivity, precision and stability of solutions.

**Linearity**

For calibration and linearity, five different concentrations of the imine derivative were used in the range of 5-50 µgmL⁻¹. The linearity of the method was determined by plotting the absorbance versus concentration of drug LNP derivative. The slope (m), intercept (b), and the correlation coefficient (R²) were determined from the regression analysis.

**Percent recovery measurement**

The % recovery was calculated by added pure drug LNP with 2HNA derivative solution as % recovery = [(D_t – D_s) / D_a] × 100 where D_t is the total drug concentration after standard addition; D_s is the drug concentration in the imine derivative mixture and D_a is the drug concentration added.

**Sensitivity**

The sensitivity of the proposed method was calculated by a limit of detection (LOD) and lower limit of quantification (LOQ) of imine derivative using signal to noise ratio (σ/s) of 3.3 σ /s and 10 σ /s, respectively;
where $\sigma$ is the standard deviation of the signal and $s$ is the slope of a corresponding calibration curve.

**Precision**

The imine derivative solution was analyzed at three intervals a day at 08:00, 16:00, 24:00, h for repeatability and for three consecutive days for reproducibility in order to assess the intermediate precision (intra-day and inter-day). The outcome was expressed as the mean ± SD and percent relative standard deviation (%RSD).

**Results and Discussion**

The motive of this research work was to develop a simple approach for the determination of LNP in pure and pharmaceutical formulations. Scheme 2 illustrates the reaction mechanism of preparation of new imine derivative LNP-2HNA by primary amino group of LNP drug with derivatizing reagent 2HNA.

![Scheme 2. Proposed reaction pathway for derivatization of drug to complex formation (LNP-2HNA)](image-url)
The 2HNA was used as a derivatizing reagent for the colorimetric analysis of LNP. The reagent 2HNA reacted with the drug LNP to produce imine derivative LNP-2HNA having a light yellow color. This reaction took place in the acid medium at pH 5.5 with maximum absorbance at ($\lambda_{\text{max}}$) 433 nm, molar absorptivity of $9 \times 10^3$ mole$^{-1}$cm$^{-1}$. The specific parameters were optimized, which effect the preparation of the 2HNA-LNP derivative similarly effect of reagent quantity, pH, heating time and temperature.

**Analytical Parameters Optimization**

**Selection of wavelength**

The wavelength of maximum absorbance shows a vital role for quantitative determinations. It is crucial to choose the wavelength where the derivative gives optimal absorbance. The absorbance of 20 μg/mL of LNP and 2HNA derivative was measured within the range of 350-500 nm. The ($\lambda_{\text{max}}$) is optimized in the visible range at 433 nm against a reference.

**Selection of optimal temperature and heating time for the preparation of derivative**

Initially, it was observed that the rate of reaction was very slow at room temperature, therefore the mixture contents were heated and the derivatization reaction was monitored on the optimal wavelength ($\lambda_{\text{max}}$) 433 nm for 0-30 min with an interval of 5 min at 95 °C.

It was observed that the best derivatization occurred by heating the reaction mixture for 15 min at 95 °C ± 1°C (Fig. 2).

**Optimization volume and concentration of reagent**

The effect of adding different quantities of reagent 2HNA solution to 1mL of drug LNP (0.02% w/v) was also studied. The reagent concentration of (1% w/v) 2HNA was varied between 0.5-3.0 mL in the 10 mL volumetric flask containing 1mL of drug LNP. There was no change in rising absorbance noticed after the addition of 2 mL reagent. Therefore, the best absorbance was measured by adding 2 mL of reagent 2HNA as shown in Fig. 3.

**pH effect on derivative**

At the most optimal conditions, the effect of adding 1 mL of 0.1 M different buffer solutions at pH ranges 2-10 was studied on the derivative. The consistent increase in absorbance was examined from pH 4-6. Further pH was specified by using buffer solution at a difference of 0.5 like pH 5.0, 5.5, 6.0, etc. The best maximum absorbance was obtained utilizing acetate buffer solution at pH 5.5 (Fig. 4). The addition of other buffers pH 8-10 revealed precipitation. Thus acetate buffer pH 5.5 was considered as optimal.
The effect of solvents on derivative was investigated by the addition of 2 mL of mentioned solvent and compared with 2 mL methanol. The procedure of determining solvent effect explained that the mixture contents containing 1 mL of drug LNP (0.02% w/v), 2 mL reagent 2HNA (1% w/v) and 1 mL acetate buffer pH-5.5, were heated for 15 minutes, then cooled same at room temperature 25 °C, then 2 mL of following mentioned solvents were added in 10 mL volumetric flask and in the blank. It was observed that none of the following solvents interfered in the LNP-2HNA derivative (Table 1).

**Table 1. Effect of solvents on derivative in terms of maximum absorbance.**

| Solvent Name   | Volume (mL) added | Abs. with methanol LNP-2HNA derivative | Abs. with other solvents LNP-2HNA derivative |
|----------------|-------------------|----------------------------------------|-------------------------------------------|
| THF            | 2                 | 0.422                                  | 0.425                                     |
| Acetone        | 2                 | 0.424                                  | 0.426                                     |
| n-Hexane       | 2                 | 0.421                                  | 0.418                                     |
| Ethyl acetate  | 2                 | 0.423                                  | 0.420                                     |
| Isopropanol    | 2                 | 0.418                                  | 0.418                                     |
| Acetonitrile   | 2                 | 0.425                                  | 0.421                                     |
| Propanol       | 2                 | 0.423                                  | 0.420                                     |
| Butanol        | 2                 | 0.422                                  | 0.425                                     |

**Effect of mixing order of reagents**

Various mixing orders in the current work were applied. The absorbance decreased when mixed 1 mL acetate buffer pH-5.5 in drug LNP (0.02% w/v), then reagent 2HNA (1% w/v). Altering the sequence of mixing by adding 2HNA first, then buffer followed by LNP solution also has revealed little amount of absorbance. It was confirmed that the addition of 1 mL of drug LNP drug first, then 2 mL reagent 2HNA followed by 1 mL buffer pH-5.5 solution provided maximum absorbance of derivative.

**Effect of additives**

The effect of the possible presence of additives like calcium hydrogen phosphate, maize starch, mannitol, pregelantised maize starch, magnesium stearate on absorbance in the determination of drug LNP was studied. Two concentration levels, first at an equal concentration of the drug LNP (0.02% w/v), and second at 10 times the concentration of drug, did not change the absorbance significantly. Not more than ±2% change in absorbance was calculated and no any additive interfered in the derivative LNP-2HNA during the determination of LNP drug (Table 2).

**Table 2. Effect of additives on absorption of derivative.**

| Name                        | Abs. without additives (LNP-2HNA) Derivative | Abs. with additives (LNP-2HNA) Derivative |
|-----------------------------|---------------------------------------------|------------------------------------------|
|                             | Equal conc. to drug                         | 10x Conc. to drug                        |
| Calcium hydrogen phosphate  | 0.420                                       | 0.422                                   |
| Maize starch                | 0.421                                       | 0.424                                   |
| Mannitol                    | 0.422                                       | 0.418                                   |
| Pregelantised maize starch  | 0.423                                       | 0.420                                   |
| Magnesium stearate          | 0.418                                       | 0.416                                   |

Percent recovery from dosage form

Table 3 shows the percentage recovery of LNP-2HNA derivative from four different commercial drugs by above
mentioned method (2). The percentage recovery was found more than 98 % in all particular formulations.

Table 3. Application of proposed method on commercial drugs.

| Drug Brands | Labeled amount per tablet (mg) | Amount found per tablet | % Recovery |
|-------------|-------------------------------|-------------------------|------------|
| Corace      | 10                            | 9.81                    | 98.1       |
| Zestril     | 10                            | 10.06                   | 100.6      |
| Trupil      | 10                            | 9.93                    | 99.3       |
| Lisna       | 20                            | 19.87                   | 99.3       |

Stability of derivative

The stability of LNP-2HNA derivative was analyzed in terms of absorbance at the concentration of 20 µgmL⁻¹ LNP. There was no significant change in absorbance was evaluated within 48 h.

Calibration graph (Beer’s Law)

A linear calibration curve (Fig. 5) regarding the correlation between absorbance and different concentrations of the drug LNP was depicting linearity within the concentration range 5-50 µgmL⁻¹ of LNP with 2HNA, and correlation coefficient of 99.96% (R²=0.9996).

![Figure 5. Linearity curve of spectrophotometric determination](image)

Reproducibility /repeatability

For the stability of derivative, the assessment of interday and intraday repeatability of the procedure is an important parameter. The methanolic solution of LNP 20 µgmL⁻¹ was taken in three separate (10 mL) calibrated flasks and the method was applied as mentioned method (1). The method was repeated for three days (n=3). The average mean absorbance of intraday and interday reproducibility for imine derivative was seen as 0.264 µgmL⁻¹ and 0.8 µgmL⁻¹ with (RSD) values 0.97% and 1.6%, respectively (Table 4).

Table 4. Sensitivity comparison of proposed method for imine derivative with AB.

| Parameters                  | Imine derivative | LNP drug |
|-----------------------------|------------------|----------|
| Precision (n=3)             | Inter-day        | 0.264    |
| Intra-day                   | 0.801            |          |
| Limit of detection (LOD)    | 0.97             | 1.77     |
| Limit of quantification (LOQ)| 1.60             | 3.97     |

Validation of the proposed method

Statistical evaluations for linearity, sensitivity, percentage recovery, precision, LOD and LOQ of the proposed method were given in (Table 5). The comparative study of our developed method with previous reported spectrophotometric methods given in (Table 6), that reveals the LOD and LOQ values were smaller over other mentioned reported methods.

Table 5. Statistical evaluations of the developed method.

| Parameter                  | Observation   |
|----------------------------|---------------|
| Derivative color           | Light yellow  |
| Absorption maxima (nm)     | 433           |
| Linearity range (µg/mL)    | 5.0-50.0      |
| Molar absorptivity (L·Mol⁻¹·cm⁻¹) | 0.910⁻²      |
| Sandell sensitivity (µg/cm²/0.001 abs unit) | 5.9×10⁻²   |
| Correlation coefficient (R²) | 0.996         |
| RSD (%)                    | 0.775         |
| Slope (b)                  | 0.01          |
| Intercept (a)              | 0.0014        |
| Percentage of recovery (%) | 98.74-.9952   |
| Limit of detection (LOD) µg/mL | 0.264     |
| Limit of quantification (LOQ) µg/mL | 0.8   |
| Intra-day variation (%)    | 0.05-.97      |
| Inter-day variation (%)    | 0.07-1.60     |
Table 6. Comparison of proposed methods with existing spectrophotometric methods for the assay of LNP in pharmaceutical formulations.

| Derivatizing reagent                  | $\lambda_{\text{max}}$ (nm) | Reaction Time (min/ temp) | LOD (µg mL$^{-1}$) | LOQ (µg mL$^{-1}$) | Linear range (µg mL$^{-1}$) | References |
|--------------------------------------|-----------------------------|---------------------------|-------------------|-------------------|-----------------------------|------------|
| 1,2-naphthoquinone-4-sulfonic (NQS)  | 481                         | 5/25°C                    | 1.16              | 3.53              | 5-50                        | [9]        |
| Alizarin                             | 434                         | 7/40°C                    | 2.08              | 6.94              | 4.412-300.23                | [46]       |
| Ninhydrin                            | 600                         | 5/80°C                    | 5.587             | 18.437            | 10-150                      | [47]       |
| Phenylhydraizine                     | 362                         | 20/85°C                   | ----              | ----              | 40-200                      | [48]       |
| Ascorbic acid                        | 530                         | 15/100°C                  | 0.349             | 1.152             | 5-50                        | [49]       |
| Chloranil                            | 346                         | ----                      | ----              | ----              | 4-20                        | [50]       |
| 2-hydroxynaphthaldehyde (2HNA)       | 433                         | 15/95°C                   | 0.264             | 0.8               | 5-50                        | Current work |

Precision

Four drug formulations were being repeatedly analyzed in three successive days in order to evaluate intra-day and inter-day reproducibility for imine derivative was seen at 0.07% and 1.6%, respectively. The %RSD values lower than 2% were obtained in our studies witness that the developed method was precise (Table 5).

Accuracy

The accuracy of the proposed method has been evaluated by applying the developed method for the determination of LNP in pharmaceutical formulations. The concentration of each drug was determined from the corresponding regression equations. The obtained percentage recoveries indicate the appropriate accuracy of the proposed method. The standard addition method was also carried out to analyze the accuracy of the method. Method accuracy was assessed for the determination of the commercial tablets by adding varying amounts of the standard LNP to a certain concentration of filtrate tablet solution. The findings showed good recoveries with low RSD. Commercial formulations have been successfully analyzed for the proposed Lisinopril method and the results were compared with the reference method [8]. (Table 4). The proposed method produces good results in both raw and pharmaceutical formulations (Table 5).

Specificity

The proposed method was determined successfully for LNP without any interference from tablet excipients, as depicted in Table 2.

Conclusion

A rapid, simple and economical spectrophotometric method using inexpensive reagents was developed for determination of Lisinopril in pure and tablet form. Our method is robust in terms of reproducibility and high sensitivity. The novelty of this proposed method is to utilize first time 2HNA reagent for derivatization of LNP drug. The LOD and LOQ values are smaller over other spectrophotometric methods reported in the literature. Moreover, the synthesized LNP imine derivative is highly stable.

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