

Spectrophotometric Determination of Lisinopril in Pharmaceuticals Using Ninhydrin- a Modified Approach

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ABSTRACT

An accurate and precise spectrophotometric method is presented for the determination of lisinopril based on the formation of a yellow colour product with ninhydrin in the presence of bicarbonate with an absorption maximum at 420 nm. The reaction proceeds quantitatively at $97 \pm 1^\circ\text{C}$ in 10 min. The calibration curve is linear over the range of 5.0-50.0 $\mu\text{g/mL}$ and is described by the regression equation $A = (-) 0.0175 + 0.0079 C$ with a regression coefficient (r) of 0.9979 ($n = 6$). The calculated molar absorptivity and Sandell sensitivity values are $2.02 \times 10^3 \text{ L/mol/cm}$ and $0.219 \mu\text{g/cm}^2$, respectively. The limits of detection (LOD) and quantification (LOQ) calculated as per ICH guidelines are 0.68 and 2.07 $\mu\text{g/mL}$, respectively. The within-day accuracy expressed as relative error was better than 2.5% with precision (RSD) ranging from 1.02 to 1.93%. The between-day accuracy ranged from 1.5-3.0% with a precision less than 4%. The method was successfully applied to the analysis of three brands of tablets containing lisinopril. The results obtained were in agreement with those obtained by the BP method. Accuracy was also checked by placebo blank and synthetic mixture analyses besides a recovery study *via* standard addition procedure. The method was also used to check the stability of lisinopril in solution.

Key words: lisinopril, ninhydrin, spectrophotometry, pharmaceuticals

INTRODUCTION

Lisinopril (LNP, Figure 1) 1-[6-Amino-2-(1-carboxy-3-phenyl-propylamino)-hexanoyl]-pyrrolidine-2-carboxylic acid, is an angiotensin converting enzyme inhibitor used in the treatment of hypertension and heart failure in prophylactic treatment after myocardial infarction and in diabetic nephropathy⁽¹⁾. The official methods for the determination of LNP are potentiometric acid-base titration⁽²⁾ and HPLC⁽³⁾ using octylsilyl silica gel column at 50°C and phosphate solution-acetonitrile (96:4, v/v) as mobile phase.

Many researchers have dealt with the development of methods that quantify LNP in pure form and in tablets. Methods that include polarographic⁽⁴⁻⁶⁾ determination of rather complicated liquid chromatographic methods with UV-detection⁽⁷⁻¹²⁾ have been described for the determination of LNP when present alone⁽⁷⁻⁹⁾ or in combination with hydrochlorothiazide⁽¹⁰⁻¹²⁾. Other techniques such as gas-liquid chromatography⁽¹³⁾, densitometric HPTLC⁽¹⁴⁾, capillary electrophoresis⁽¹⁵⁾ and spectrofluorimetry^(7,16-18) though sensitive are expensive. A number of deriva-

tive UV-spectrophotometric methods^(9,14,18-25) are also found in the literature, but these methods are most applicable for relatively high concentrations and combined dosage forms.

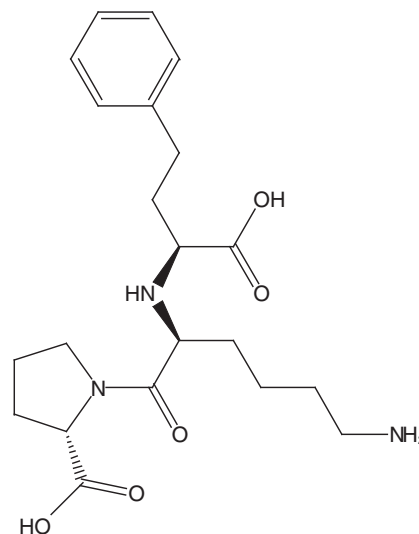


Figure 1. Structure of lisinopril

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Table 1. Comparison of current visible spectrophotometry methods

Sl. No.	Reagent/Reaction	Range μg/mL	ϵ , L/mol/cm	LOD	Remarks	Ref. No.
1.	Reaction of the LNP with sodium hypochlorite and phenyl hydrazine to form a condensation product. λ_{\max} =362 nm			NA	Measurement at a shorter wavelength	7
2.	a. Reaction between LNP and chloranil in aqueous solution of pH 9.5. λ_{\max} =346 nm b. LNP with dichlone, formation of purple colour. λ_{\max} =580 nm c. Condensation reaction with acetylacetone and formaldehyde. λ_{\max} =356 nm				pH dependent	18
3.	a. Reaction between LNP and N-bromosuccinimide in acetone. λ_{\max} = 353 nm b. Charge transfer complex reaction between LNP and chloranil in 1,4-dioxan-acetonitrile.	10-200 24-600	1.4×10^3 7.28×10^2	NA	Less sensitive, use of organic solvents, measure at shorter wavelength.	26
4.	a. Charge transfer complex reaction between LNP and 7,7,8,8 tetracyanoquinodimethane in polar media. λ_{\max} =743 nm b. Charge transfer complex reaction between LNP and p-chloranilic acid in polar media. λ_{\max} = 525 nm	2-26 25-300	1.432×10^4 1.192×10^4	NA	Expensive chemicals used, and use of organic solvent.	27
5.	LNP derivatization reaction with 1-fluoro-2,4-dinitrobenzene in acetonitrile at pH 8.2 in dark at 60°C for 45 min, follow by acidification with HCl. a. λ_{\max} = 356.5 nm b. λ_{\max} = 405.5 nm	20-120		0.014 mg/L 0.023 mg/L	Less sensitive	28
6.	a. Two kinetic methods were described based on the reaction of LNP with ninhydrin in DMF medium at RT. λ_{\max} =595 nm (1). Initial-rate method (2). Rate-constant (10 min) method b. LNP reacts with ascorbic acid in DMF medium. λ_{\max} =530 nm	10-50 10-40 5.0-50.0	4.55×10^3	NA	Kinetic studies; organic solvent used, rate is critically dependent on experimental variables.	29
7.	Reaction between LNP and ninhydrin in the presence of DMF. λ_{\max} =600 nm	10-150	4.083×10^3	NA	Organic solvent used	30
8.	Reaction between LNP and ninhydrin in acetone. λ_{\max} =410 nm	10-40	NA	NA	Organic solvent used	31
9.	Reaction between LNP and ninhydrin in aqueous medium in the presence of saturated NaHCO_3 . λ_{\max} =420 nm	5.0-50.0	2.02×10^3	0.68 μg/mL	More greener method	This work

NA: Not Available.

Visible spectrophotometry is the technique of choice even today because of its inherent simplicity, sensitivity, selectivity, accuracy, precision and cost-effectiveness. LNP in pharmaceuticals has been assayed based on reaction with N-bromosuccinimide and cloram⁽²⁶⁾, chloranil, dichlone and acetyl acetone-HCHO⁽¹⁸⁾, π -acceptors⁽²⁷⁾ sodium hypochlorite-phenylhydrazine⁽⁷⁾, 1-fluoro-2,4-dinitrobenzene⁽²⁸⁾ and ascorbic acid⁽²⁹⁾. Most of these methods

employ organic solvents as reaction medium, require longer heating times, use expensive reagents, and/or are less sensitive (Table 1). Of the various reagents used in the assay of LNP in pharmaceuticals, ninhydrin has been employed by quite a few researchers. For example, Rehman *et al.*⁽²⁹⁾ used ninhydrin in DMF medium for kinetic spectrophotometric determination of LNP by initial rate and fixed-time procedures. Both methods showed linear response over 10-

50 µg/mL LNP. The reagent in the same organic solvent medium (DMF) but involving heating was used by Raza *et al.*⁽³⁰⁾ to quantify LNP in 10-150 µg/mL range. Rajashekar and Udayavani⁽³¹⁾ assayed LNP in the 10-40 µg/mL range by measuring the coloured product formed between ninhydrin and LNP in acetone medium at elevated temperature. The common feature of all the three methods using ninhydrin⁽²⁹⁻³¹⁾ is the use of organic solvent as the reaction medium which quite often is undesirable.

The scientific references found in the CAS and SCI database, relating to green analytical chemistry or environmentally-friendly analytical methods have been growing significantly in recent years⁽³²⁾. The recent development of new analytical methods with good characteristics such as selectivity and sensitivity are not sufficient; modern analytical methods need to be green^(33,34). Hence, the purpose of the present study was to improve these methods with respect to two experimental objectives: (i) decrease the heating time, (ii) avoid the use of organic solvent. Both factors are of great significance in reducing time and cost of analysis. In the present study, sodium bicarbonate was used as the reaction medium and no organic solvent was employed. In addition, the heating time was relatively short (10 min) compared to the reported methods using ninhydrin⁽²⁹⁻³¹⁾. These modifications resulted in increased linear range (5-50 µg/mL) and sensitivity ($\epsilon = 2.02 \times 10^3$ L/mol/cm) compared to many existing spectrophotometric methods.

MATERIALS AND METHODS

I. Apparatus

A Systronics model 106 digital spectrophotometer provided with 1 cm matched quartz cells was used for absorbance measurements.

II. Reagents and Materials

All chemicals were of analytical reagent grade and distilled water was used to prepare solutions.

(I) 0.5% Ninhydrin

500 mg of chemical (Merck, Mumbai, India) was dissolved in water and made up to 100 mL with water.

(II) Sodium Bicarbonate (Saturated Solution)

Approximately 25 g of the chemical (Loba chemie, Mumbai, India) was taken in a beaker containing 100 mL of water and stirred with a magnetic stirrer for twenty minutes. The solution was decanted and filtered using quantitative filter paper.

(III) Standard Drug Solution

Pharmaceutical grade LNP was kindly provided by Cipla Pvt. Ltd., India and was used as received. A stock standard solution equivalent to 500 µg/mL LNP was prepared by dissolving 50 mg of pure drug in water and diluting to 100 mL in calibrated flask with water. The stock solution was diluted appropriately to reach working concentration of 100 µg/mL with water.

III. Methods

Different aliquots (0.0, 0.5,.....5.0 mL) of 100 µg/mL LNP solution were accurately measured and transferred into a series of 10 mL volumetric flasks. The total volume was brought to 5 mL with water. One mL of 0.5% ninhydrin and then 1 mL of saturated solution of NaHCO₃ were added to each flask, and the total volume was brought to 9 mL with water. All flasks were kept in a boiling water bath for 10 minutes, and cooled to room temperature before making up to the mark with water. Absorbance was measured at 420 nm *versus* reagent blank. A calibration graph was prepared by plotting the measured absorbance *versus* concentration. The concentration of the unknown solution was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

IV. Procedure for Commercial Tablets

Twenty tablets were weighed accurately and ground into a fine powder. Powder equivalent to 10 mg of LNP was weighed accurately and transferred into a 100 mL volumetric flask with 60 mL of water. The content was shaken for 15-20 min, diluted to volume with water, and filtered using a Whatman No. 42 filter paper. First 10 mL portion of filtrate was discarded and subsequent portions were subjected to analysis.

RESULTS AND DISCUSSION

Ninhydrin forms purple color product with primary amine, which is the basis for quantification of compounds containing primary amino groups. All reactions were carried in organic solvents such as DMF, DMSO, acetone or methanol. The present authors found that ninhydrin forms yellow color product with LNP, having both primary and secondary amines, in the presence of saturated solution of NaHCO₃ and without employing any organic solvents. The blank showed higher absorbance at 340 nm compared at 420 nm for a given ninhydrin concentration (Figure 2). The blank absorbance was negligible at 420 nm, and hence all absorbance measurements were made at 420 nm against reagent blank instead of 340 nm. The yellow color product forms slowly in ambient temperature (nearly 5-6 hours), whereas it takes only ten minutes for reaction to complete when the reaction flask was kept in a boiling water bath.

I. Optimization of Reaction Conditions

Reaction between ninhydrin and LNP did not give any colored product in the absence of NaHCO_3 , not even after prolonged heating. Four parameters, volume of NaHCO_3 , effect of pH, concentration of ninhydrin and boiling time, were optimized. When more than 1 mL of saturated solution of NaHCO_3 was used, a violet color product resulted, which absorbs maximally at 480 nm (Figure 3). But the violet color product was unstable and turned yellow with time (absorption maximum at 420 nm). Hence, 1 mL of saturated solution of NaHCO_3 , resulting in the formation of stable yellow color product, was the optimum in a total volume of 9 mL. Interestingly, reaction was found to be specific in NaHCO_3 medium. Different concentrations of NaOH were used to study the effect of pH on the reaction between ninhydrin and LNP. Since no color product was formed, the reaction is specific in bicarbonate medium. Aqueous solution of ninhydrin is yellow which absorbs appreciably at 420 nm. In order to maintain the lowest absorption due to ninhydrin at 420 nm and to provide sufficient ninhydrin concentration to react with LNP, change in absorbance due to varying concentration of ninhydrin on fixed concentration of LNP (50 $\mu\text{g/mL}$) were measured against respective reagent blank (Figure 4). Either 2.5 mL of 0.2% ninhydrin or 1 mL of 0.5% ninhydrin in a total volume of 10 mL was found optimum.

When the heating time exceeded 10 min, the violet color product with a maximum absorption at 480 nm became unstable, and the color kept changing to yellow. Therefore, heating time of 10 min in a boiling water bath was found optimum for the development of stable yellow color product; the developed color was stable for 90 min. All measurements were made at 420 nm against reagent blank.

II. Method Validation

(I) Linearity, Detection and Quantification Limits

A linear correlation was found between absorbance and concentration of LNP. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and the correlation coefficient (r) (Table 2). The graph shows negligible intercept and is described by the regression equation $y = a + bx$, where y is the absorbance and x is concentration in $\mu\text{g/mL}$. The limits of detection (LOD) and quantification (LOQ), sensitivity parameters such as molar absorptivity, and Sandell sensitivity are also contained in Table 2.

(II) Selectivity

Selectivity, defined as the ability of the method to determine the analyte in the presence of matrix, was evaluated by preparing a synthetic mixture. It was confirmed that the change in measured signal (absorbance) was

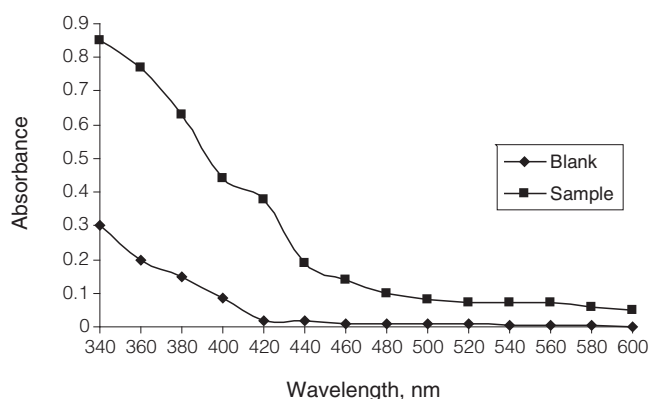


Figure 2. Absorption spectra

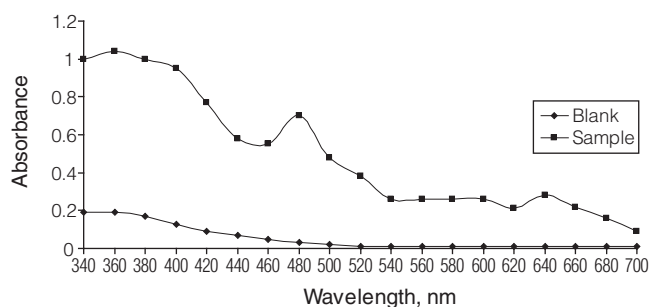


Figure 3. Absorption spectra for the unstable violet color product

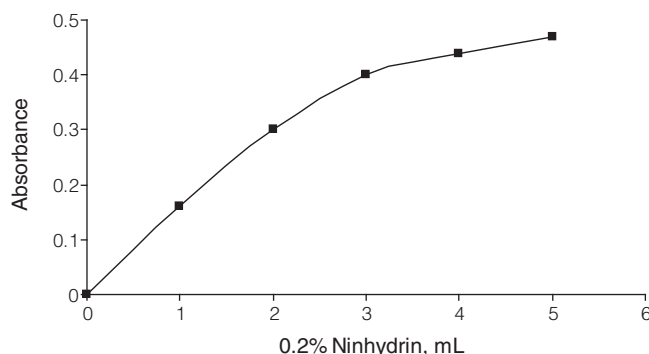


Figure 4. Effect of ninhydrin concentration

caused by only the analyte. A synthetic mixture with the composition equivalent to the amount of excipients was present in the formulations: LNP (10 mg), talc (20 mg), starch (30 mg), lactose (60 mg), gum arabic (20 mg), magnesium stearate (10 mg) and sodium alginate (15 mg). The extract was prepared according to the procedure described for tablets and analyzed. Replicate analyses ($n = 7$) of the extract by the procedure described earlier at 25 $\mu\text{g/mL}$ concentration levels indicated that the inactive ingredients did not interfere with LNP determination. This was supported by the % recovery of LNP at 101.67 ± 1.3 .

(III) Precision

Precision of the method was calculated in terms of intermediate precision (intra-day and inter-day)⁽³⁵⁾. Three different concentration of LNP were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed good precision (Table 3).

(IV) Accuracy

Accuracy of an analytical method is the closeness between the reference value and the found value⁽³⁵⁾. Accuracy was evaluated as percentage relative error between the measured concentrations and actual concentrations for LNP (Bias %). The results obtained are compiled in Table 3 and show good accuracy for the method.

(V) Application to Tablets Analysis

The proposed method was applied to determine LNP in two brands of tablets with three different doses. The

results were statistically compared with those obtained by the official British Pharmacopoeial method⁽²⁾ using the Student's t-test and variance ratio F-test. The official method consisted of titration of the aqueous solution of the tablet with 0.1 M NaOH potentiometrically. Statistical analysis using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95% confidence (Table 4).

(VI) Recovery Study

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure LNP at three concentration levels (50,100 and 150%) and the total was found by the proposed method. In all cases, the added LNP recovery percentage values ranged between 101.3 and 107.5 with relative standard deviation of 2%.

Table 2. Regression parameters and sensitivity values

Parameter	Value
Linearity range, µg/mL	5.0-50.0
ϵ , L/mol/cm	2.02×10^3
Sandell sensitivity, µg/cm ²	0.219
Slope (b)	0.0079
Intercept (a)	-0.0175
Standard deviation about regression (S_y)	± 0.0981
Standard deviation of Slope (S_b)	± 0.0025
Standard deviation of intercept (S_a)	± 0.0763
Correlation co-efficient (r)	0.9979
Limit of detection (LOD, µg/mL)	0.68
Limit of quantification (LOQ, µg/mL)	2.07

Table 4. Comparison of assay results of proposed and reference methods

Tablet brand name**	Label claim, mg/tablet	Found* (% of label claim \pm SD)	
		Reference method	Proposed method
Hipril ^a	10	98.56 \pm 0.85	99.62 \pm 1.58 t = 2.25 F = 3.45
		99.64 \pm 0.72	101.2 \pm 1.44 t = 2.28 F = 4.00
Lipril ^b	5.0		100.8 \pm 1.42 t = 1.26 F = 5.25
		101.3 \pm 0.62	
Normopril ^c	2.5		

*Mean value of five determinations

Tabulated t-value at the 95% confidence level is 2.77

Tabulated F-value at the 95% confidence level is 6.39

**Marketed by: ^a Micro Labs Ltd, Bangalore, India

^b Lupin Laboratories Ltd., Mumbai, India

^c Lupin Ltd., Jammu, India

Table 3. Evaluation of intra-day and inter-day accuracy and precision

LNP taken µg/mL	Intra-day accuracy and precision			Inter-day accuracy and precision		
	LNP found, µg/mL	RE %	RSD %	LNP found µg/mL	RE %	RSD %
30	29.89	0.34	1.93	29.53	1.57	2.56
40	40.87	-2.17	1.82	39.11	2.23	3.82
50	48.75	2.50	1.02	48.58	2.84	2.72

RE. Relative error; RSD. Relative standard deviation.

Table 5. Results of recovery study by standard-addition method

Formulation studied	LNP in tablet, µg/mL	Pure LNP added, µg/mL	Total found, µg/mL	Pure LNP recovered*, Percent ± SD
Hipril- 10 mg	19.71	10.0	30.40	103.5± 2.74
	19.71	20.0	40.91	106.1 ± 1.28
	19.71	30.0	50.03	101.6 ± 1.18
	20.26	10.0	31.78	107.5 ± 1.86
Normopril-2.5 mg	20.26	20.0	40.99	103.6 ± 2.01
	20.26	30.0	50.52	101.3 ± 1.72

* Mean value of three determinations.

The results of this study given in Table 5 indicated good recovery and no interference in the determination of the co-formulated substances.

CONCLUSIONS

The new spectrophotometric method developed for the determination of LNP uses readily available and inexpensive chemicals compared to many reported methods. The method is selective, reproducible and inexpensive. A comparison of the proposed procedure with other reported methods show better sensitivity and more attainable reagents than many methods (Table 1). In comparison with the existing visible spectrophotometric methods for the quantification of LNP, the present modified method can be considered green as it demonstrates that visible spectrophotometry can be utilized without the usage of organic solvent. Compared to the BP method⁽²⁾, the presented method is more sensitive since the former requires 350 mg of drug for each titration. Both US Pharmacopeia⁽³⁾ and European Pharmacopoeia⁽³⁶⁾ describe HPLC procedure for assay in tablets. Both seem to be less sensitive in comparison with the proposed method since USP and EP procedures require 300 µg/mL and 2000 µg/mL of sample solution, respectively. In addition, the proposed method employs an inexpensive instrument compared to both USP and EP procedures.

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