SUNTEXT REVIEWS

SunText Review of Pharmaceutical Sciences
ISSN: 2766-5232

Open Access
Research Article
Volume 7:1

Development and Validation of UV Spectrophotometric Method for Determination of Lisinopril in Bulk and Pharmaceutical Dosage Forms Using Vanillin as Chromogen

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Received date: 05 April 2026; **Accepted date:** 02 May 2026; **Published date:** 10 May 2026

Citation: Adam ME, Shaa Eldean MEA, Fatehalrahman FM, Abdelrahaman AN. (2026). Development and Validation of UV Spectrophotometric Method for Determination of Lisinopril in Bulk and Pharmaceutical Dosage Forms Using Vanillin as Chromogen. SunText Rev Pharm Sci 7(1): 138.

DOI: https://doi.org/10.51737/2766-5232.2026.038

Editorial

A simple, sensitive, selective and rapid UV spectrophotometric method was described for the determination of lisinopril in Bulk drug and in dosage forms using Vanillin as the chromogenic agent. The method is based on the condensation reaction between primary aromatic amine group present in lisinopril with aromatic Aldehyde, vanillin to produce an intense yellow colored product. The Resulting Schiff's base shows an absorption maximum at 395 nm. The different affecting formation and stability of the complex were carefully studied. The reaction was carried out in methanol under heat condition reaction. The method was validated for linearity, range, accuracy and precision. The calibration curve was linear (R2= 0.9996) in the concentration ranges 20-180 µg/ml lisinopril. The limit of detection was found to be = 8.236µg/ml, while the limit of quantification was =24.958 µg/ml. The RSD% values for the three precision levels were (< 2). The recoveries (n=3) were 98.3%%, 100.3%% and 100.8%% for 50% ,100% and 150% levels respectively. The method was successfully adopted to determine the content percent of lisinopril in two tablet brands marketed in Sudan.

Key words: Lisinopril; Vanillin; UV spectrophotometric method

Introduction

Lisinopril chemically, is I(S)-1-[N2-(1-carboxy-3-phenylpropyl)-L¬lysyl]-L-proline dihydrate (Figure 1). It is a synthetic peptide derivative, that act oral long-acting angiotensin converting enzyme inhibitor. Lisinopril is used for management of hypertension and heart failure [1]. Vanillin (4-hydroxy-3-methoxybenzaldehyde) (Figure 2) is the member of the class of Benzene aldehydes carrying methoxy and hydroxyl substituent at position 3 and 4 respectively. Vanillin, as a component of vanilla, used as a flavouring agent in foods and beverages. It has many medicinal uses such as anticancer [2], antidiabetic [3] antioxidant [4], antibacterial [5] and antidepressant properties [6]. The official method of assay of lisinopril in USP using HPLC at wavelength 210nm and BP determining lisinopril titrimetric method, the end-point detected potentiometrically. Literature review reveals many methods for determination of lisinopril in pharmaceutical formulation. They include:

Spectrophotometric method [7-12]. The HPLC methods for analysis of Lisinopril alone or combined with another drugs [13-18]. Most of the reported method lacking selectivity or complicated and expensive instruments there is a need for simple selective

method for analysis of Lisinopril in bulk and dosage form. In this report UV spectrophotometric method for estimation of Lisinopril using Vanillin as chromogen was described.

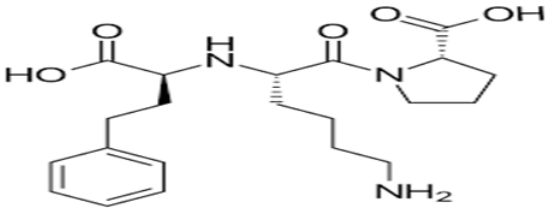


Figure 1: *chemical structure of lisinopril.*

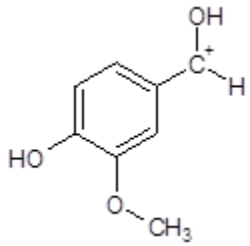


Figure 2: *Chemical Structure of Vanillin.*

Materials and Methods

Materials

Drugs

Lisinopril standard (Working STD) certifies from AZAL
Lisinopril tablet dosage forms (Azapril 10mg and Linopril 10mg)

Reagents

Vanillin (vanillin 99%) LOBA CHMIE PVT.LTD
Phosphoric acid
Sodium 1-hexanesulfonate
Monobasic potassium phosphate
Methanol
Acetonitrile
Instruments
UV-VIS Spectrophotometer (SHIMADZU 1800)
HPLC SHIMADZU Prominence

Methods

Preparation of Reagents

Preparation of Vanillin solution

Vanillin solution was prepared by dissolving 4gms of vanillin in 100ml of methanol.

Preparation of lisinopril 200µg/ml standard solution

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added. The solution was sonicated for ten minutes and the solution was completed to volume withe methanol to obtain a solution of concentration of about 200µg/ml.

Preparation of lisinopril 100µg/ml standard solution

Dilute standard stock solution 5ml to 10ml VF with methanol to obtain a solution having a known concentration of about 100µg/ml

Preparation of lisinopril sample stock solution

Dissolve an accurately weighed quantity of Powder tablet containing 20mg lisinopril in methanol to obtain a solution having a known concentration of about 200 µg/ ml heat into water bath at 600c for about 30minutes.

Scanning of lisinopril standard solution

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added. The solution was sonicated for ten minutes and the solution was completed to volume withe methanol to obtain a solution of concentration of about 200µg/ml. The solution was heated on to water bath at 60°c for 30 minutes. The solution was scanned between 200 -800nm using methanol as blank.

Scanning of Vanillin standard solution

Vanillin solution was prepared by dissolving 4gms of vanillin in 100ml of methanol. The solution was heated on to water bath at 60oc for 30minutes. The solution was scanned between 200 -800nm using methanol as blank.

Preparation of lisinopril and vanillin mixture and development of colored complex

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added. The solution was sonicated to dissolve and add 2ml of vanillin 10% W/V then complete to volume using methanol. The mixture was heated on to water bath at 60oc for 30minutes. The solution was scanned between 200 -800nm using methanol as blank.

Effect of solvent on formation of the lisinopril-vanillin Complex

Four solutions of mixture of lisinopril-vanillin were prepared in different solvents mainly Dimethylformamide (DMF), Dimethyl sulfoxide (DMSO), methanol and ethanol as follows: Weight (5mg) of standard lisinopril was transferred to 50ml volumetric flask and dissolved in the minimum volume of methanol with aid of sonication, 2ml of vanillin 10% W/V and the was completed to 50ml using Dimethylformamide. Three other solutions were prepared similarly using. methanol, Dimethyl sulfoxide and ethanol. Each solution was heated on water bath at 60oc for

30minutes. The solution was scanned between 200 -800nm using the respective solvent as blank.

Effect of temperature on formation of the lisinopril-Vanillin Complex

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added. The solution was sonicated to dissolve and add 2ml of vanillin 10% W/V then complete to volume using methanol. Aliquot volume of the mixture (30ml) was heated on to water bath at 25,30,40,40, 50 and 60oc each for 30minutes. Each solution was cooled scanned between 200 -800nm using methanol as blank

Method Validation

Linearity

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added, the solution was sonicated for two minutes and the solution was completed to 100ml. Serial volumes (1-10ml) were each was transferred to 10ml volumetric flask, 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60^{o}c for 30 minutes. The absorbance of each was measured at 395nm. The calibration curve was constructed by plot of absorbance v.s concentration. The linearity of the method was verified by calculation of correlation coefficient r2 and Limit of detection (LOD) and limit of quantification (LOQ) according to the following relationship

LOD= 3.3 σ/S

where

σ = the standard error of the response

S = the slope of the calibration curves the slope

LOQ = 10 σ/S

where

σ = the standard deviation of the response

S = the slope of the calibration curve.

Precision

Repeatability

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added, the solution was sonicated for two minutes and the solution was completed to 100ml. Serial volumes (3ml) were each was transferred to 10ml volumetric flask, 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60^{o}c for 30 minutes.

The absorbance of each was measured six times at 395nm. The mean, standard deviation and RSD were calculated

Intraday precision

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added, the solution was sonicated for two minutes and the solution was completed to 100ml. Serial volumes (3ml) were each was transferred to 10ml volumetric flask, 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60^{o}c for 30 minutes. The absorbance of each was measured three times days (initially, after 3hours and six hours) at 395nm. The mean, standard deviation and RSD were calculated

Interday precision

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added, the solution was sonicated for two minutes and the solution was completed to 100ml. Serial volumes (3ml) were each was transferred to 10ml volumetric flask, 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60oc for 30 minutes. The absorbance of each was measured in three consecutive days at 395nm. The mean, standard deviation and RSD were calculated

Accuracy

Preparation of lisinopril standard solution

Accurate weight of Lisinopril WS (20mg) was transferred to 100ml volumetric flask, 70ml of methanol were added, the solution was sonicated for two minutes and the solution was completed to 100ml. Three volumes (5,10 and 15ml) were each was transferred to 10ml volumetric flask, 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60oc for 30 minutes. The absorbance of each solution was measured at 395nm using methanol as blank.

Preparation of lisinopril sample solution

Twenty tablets of azinopril brand were weighed and powdered. Powder tablet containing 20mg lisinopril was weighed and transferred to 100ml volumetric flask, 70ml of methanol were added. The solution was sonicated for ten minutes and the solution was completed to volume withe methanol. The solution was filtered. 10ml. was transferred to 10ml volumetric flask, 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60oc for 30 minutes. The absorbance of each solution was measured at 395nm using methanol as blank.

Preparation of lisinopril mixture solution

From the filtrate in (2.3.3.2), aliquot volumes(10ml) each was transferred to 10 ml flask numbered 1,2,3 then 5,10 and 15ml from

solution in 2.3.3.1 respectively. 2ml of 10% w/v vanillin were added and the volume was completed to 10ml volumetric flask using methanol. Each solution was heated on water bath at 60oc for 30 minutes. The absorbance of each solution was measured at 395nm using methanol as blank. The recovery percent was calculated for each level.

Determination of content % of lisinopril in tablet brands using the proposed methods

Weigh and powder 20 tablets of each of the two tablet brands. Dissolve an accurately weighed quantity of Powder tablet containing 20mg lisinopril of each brand and transferred to100ml volumetric flask. Add 70ml methanol, the solution was sonicated and volume was completed to 100ml using water. A portion of the reaction mixture was filtered. Aliquot volume of the filtrate(1ml) was transferred to 10ml volumetric flask, 2mls of 10%w/v vanillin was added and the volume was completed to 10ml with methanol. Each solution was heated on water bath at 600c for about 30minutes. The solution was cooled to room temperature and absorbance of each solution was measured at 395nm. The absorbance of each sample was incorporated in the regression to calculate the actual concentration of lisinopril in each brand powder.

The content percent = actual concentration/theoretical actual concentration X 100%

Determination of content percent of lisinopril in tablet brands using the official method USP test method of tablet Assay

Phosphate solution

Dissolve 4.1 g of monobasic potassium phosphate in about 900 mL of water in a 1000-mL volumetric flask, and adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and mix.

Mobile phase:

Dissolve 1.0 g of sodium 1-hexanesulfonate in 820 mL of Phosphate solution. Add 180 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see System Suitability under Chromatography ⟨621⟩).

Diluent:

Prepare a mixture of water and methanol (4:1).

Standard preparation:

Dissolve an accurately weighed quantity of USP Lisinopril RS in Diluent to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation:

Transfer to a suitable size volumetric flask 10 Tablets, which when diluted with Diluent will yield a solution having a concentration of about 0.2 mg per mL. Add Diluent, and sonicate for 5 minutes. Shake the flask by mechanical means for 20 minutes, dilute with Diluent to volume, mix, and filter.

Chromatographic system

(see Chromatography ⟨621⟩) The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 20-cm column that contains packing L7 and is maintained at a temperature of 40°. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; the capacity factor, k′, for the analyte peak is greater than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

Procedure:

Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of C21H31N3O5 in each Tablet taken by the formula: (L/D) x C x(rU/rS) in which L is the labeled quantity, in mg, of lisinopril in each Tablet, D is the concentration, in mg per mL, of lisinopril in the Assay preparation based on the labeled quantity per Tablet and the extent of dilution; C is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the Standard preparation; and rU and rS are the lisinopril peak area responses obtained from the Assay preparation and the Standard preparation, respectively.

Molar Ratio Method

A weight of (10mg) of lisinopril standard was taken, transferred to 100 ml flask ,70ml of methanol was added. The solution was shaken and the was made to 100ml using methanol. Six volumes of this solution(ml), each was transferred to 10ml flask then 0.5, 1, 1.5, 2, 2.5, 3, 5ml of 150µg/ml of Vanillin solution were added respectively. and the volume was completed to 10ml with methanol. Each solution was heated on water bath at 60^0c for about 30minutes. The absorbance of each solution was measured at 395nm using methanol as blank. The ratio lisinopril/vanillin was plotted against absorbance of the mixture.

Results and Discussion

Lisinopril is a synthetic peptide derivative; it is used for treatment of hypertension and heart failure appear to result primarily from suppression of the renin- angiotensin-aldosterone system. Colorimetry is a technique which involves the quantitative estimation of colors frequently used in biochemical investigation. Color can be produced by any substance when it binds with color forming chromogens. The difference in color intensity results in difference in the absorption of light. The intensity of color is directly proportional to the concentration of the compound being

measured.1 Wavelength between 380 nm to 780 nm forms the visible band of light in electromagnetic spectrum. Vanillin is the member of the class of Benz aldehydes carrying methoxy and hydroxyl substituent at position 3 and 4 respectively. It has application in colorimetric measurement of drugs in dosage forms and biological fluid as chromogen [19-28]. Simple UV spectrophotometric method for assay of lisinopril in bulk and in tablet dosage forms was developed. The method was based on reaction between the primary in lisinopril and aldehydic group of vanillin. The product has a yellow colour of absorption maxima at 395nm upon heating the reaction mixture at 60oc for 30minutes. Methanol is used as solvent (Figure 3). When the same concentration of lisinopril and vanillin solutions were heated individually at 60c and for 30minutes no color developed and they do not show any absorption maxima in visible region (Figure 4,5).

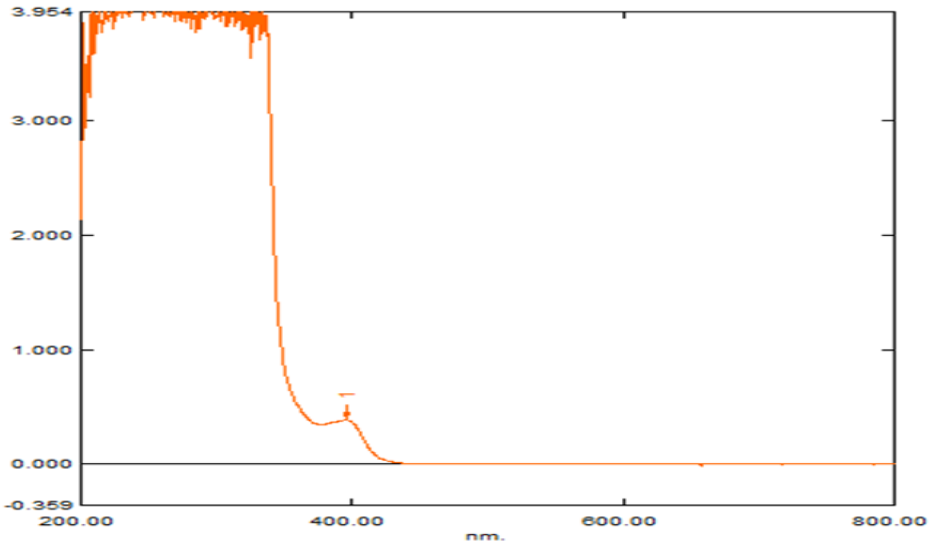
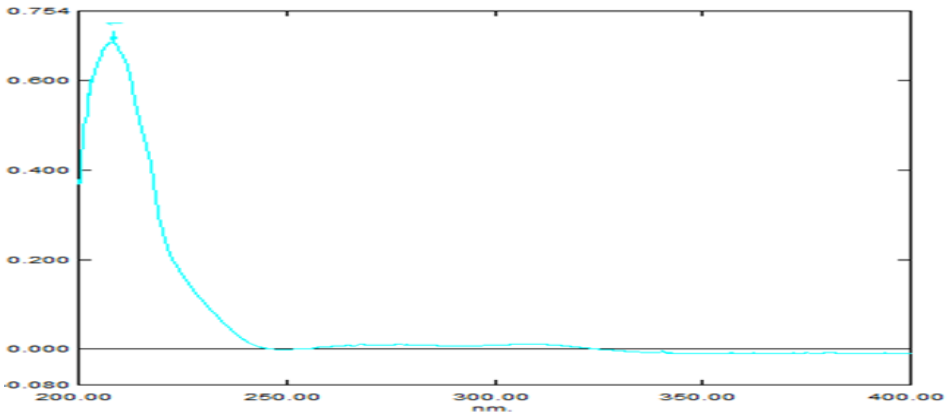


Figure 3: *Spectrum of lisinopril and vanillin mixture.*



Linearity

Linearity of the method was checked by measuring the absorbance of serial solutions prepared by solution A and plot the absorbance vs. concentrations and it was found to be linear(R=.9996), Y= 0.0.0049x+ 0.0481 in the range of 20-180 µg/ml. fig 4 where y is absorbance of the complex and c is concentration of Lisinopril standard solution. Regression analysis data of the developed method was summerized in (Table 1) (Figure 6).

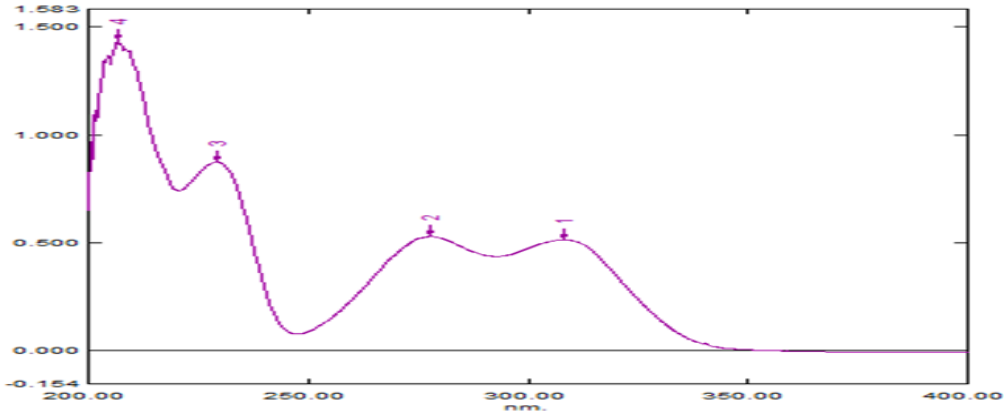


Figure 5: *UV Spectrum of vanillin in methanol.*

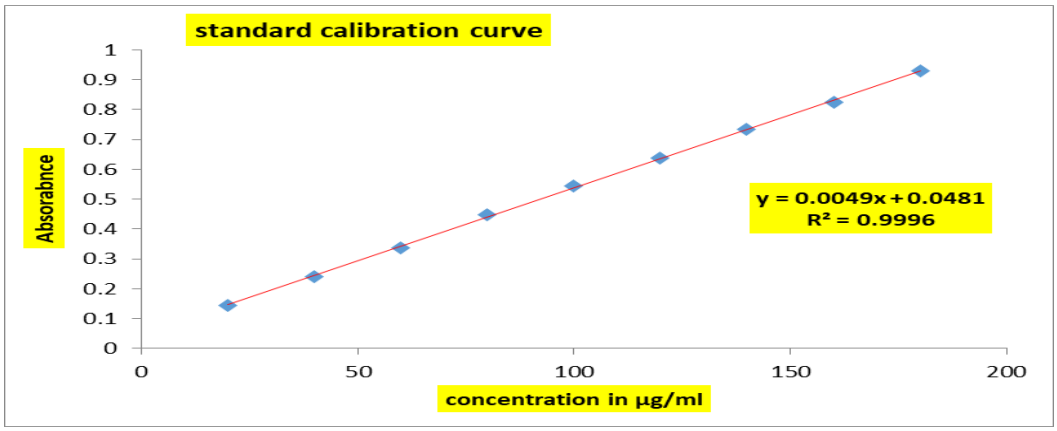


Figure 6: *Calibration curve of the proposed method.*

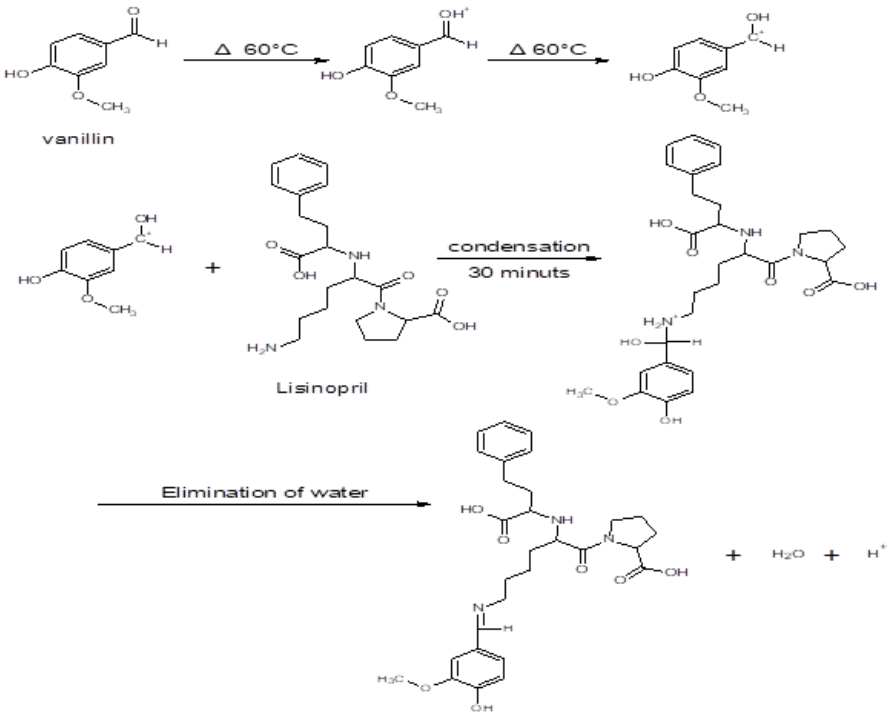


Figure 7: *Proposed Lisinopril-Vanillin reaction mechanism.*

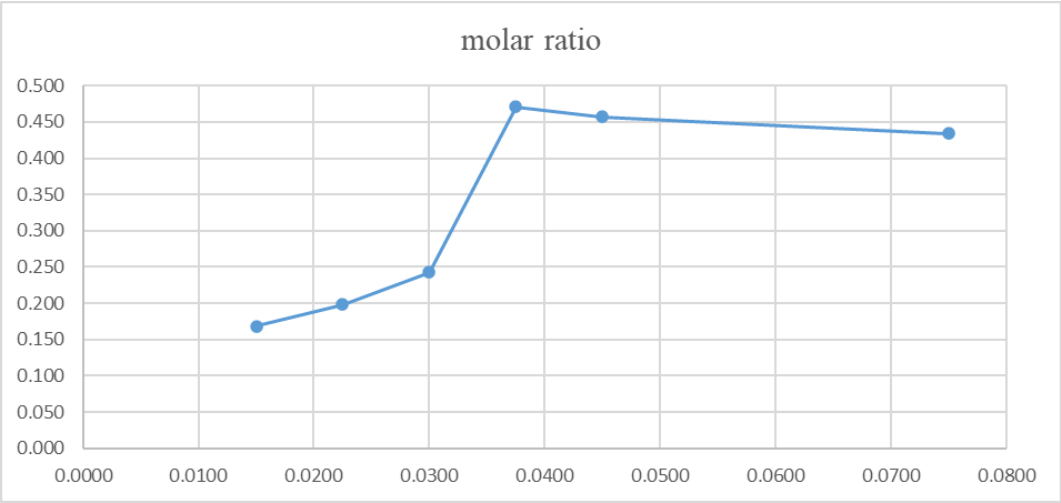


Figure 8: *Molar ratio of Lisinopril reaction with Vanillin (drug/reagent).*

Table 1: *Regression analysis data of the developed method.*

Parrameter	Developed method
Range	20-180 µg/ml
R^2	0.9996
Slope	0.0049
Intercept	0.0481
LOD	8.236 µg/ml
LOQ	24.958 µg/ml

Table 2: *The accuracy studies of the proposed method.*

Run	Sample	Standard	Mixture	Difference	Recovery%
	0.257	0.252	0.513	0.256	99.6
	0.257	0.492	0.750	0.493	100.2
	0.257	0.735	0.998	0.741	100.8

Table 3: *Content percent of Lisinopril of in tablet brands using the proposed Method.*

Brand	Absorbance of standard	Absorbance of standard	Content percent
Azapril	0.477	0.491	102.9
linopril	0.541	0.555	102.6

Table 4: *Content percent of Lisinopril of in tablet brands using the official Method (HPLC).*

Brand	Peak of the standard	Peak area of the sample	Content percent
Azapril	5992629	5868802	97.9
linopril	5992629	5976932	99.7

Precision

Method precision was assessed in term of repeatability, intra-day and inter-day precision by measuring the absorbance of solution of Lisinopril six times in the same for repeatability, at three-time intervals in the same day for intra-day and in three different days for inter-day precision. The RSD values were 1.36 for repeatability, 1.5% for intra-day precision and 1.21% for inter-day precision respectively. The small RSD values obtained (< 2) reflects the precision of the developed method.

Accuracy

The accuracy of the develop method was determined standard addition method calculating the percent recoveries of Lisinopril by addition of known amount of standard drug in the pre-analyzed injection formulation .in 50% ,100% and 150% the recovery % were 99.6%, 100.2% and 100.8% respectively (Table 2). These results reflect the accuracy of the develop method and its freedom from interference of excipients.

Content percent of Lisinopril in tablet brands using both the proposed and the official methods

The content percent of Lisinopril in tablet of the two brands was calculated using the proposed method and the official (HPLC) method. The results were presented in (Tables 3,4) respectively. Both brands showed content percent of lisinopril with the limit of the official range.

Proposed Mechanism of the reaction

As proposed in fig 5, the reaction proceeds by nucleophilic addition. The electron lone pair of the amino group in Lisinopril attacks the most electrophilic carbonyl carbon in Vanillin to give an imine which undergoes hydrolysis into an amine. The amine then loses water molecule and proton to give the colored complex. This proposed mechanism is confirmed by the stoichiometry results of molar ratio [drug: regent] to be 1:1 (Figures 7,8).

Conclusion

The developed method was proved to be simple, accurate and precise for the determination of Lisinopril in bulk and dosage forms. Vanillin is considered a suitable, cheap and available reagent for the analysis of Lisinopril. The developed method can be used for the routine analysis of Lisinopril in quality control laboratories. The developed methods can be modified in order to apply for analysis of lisinopril in biological samples.

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