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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF LISINOPRIL IN PHARMACEUTICAL FORMULATION

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ABSTRACT:

Three simple and sensitive spectrophotometric methods for the determination of lisinopril in tablets are developed. The first method involves oxidation of lisinopril with 1-chlorobenzotriazole reagent (CBT) in strong alkaline medium followed by measuring the absorbance at 350 nm. The method obeys Beer's law over concentration range 8-64 μg ml^{-1} with mean recovery 99.28±1.088. The second method involves the reaction of the primary amino group of lisinopril with ninhydrin reagent producing a coloured product with absorbance maximum at 564 nm. The linearity range was found to be 4 - 20 $\mu g ml^{-1}$ with mean recovery 99.094±1.069. The third method is based on the coupling reaction with 1,2-naphthaquinone-4-sulphonate (NQS) to form a coloured product (λ_{max} 305). Regression analysis of a Beer's plot showed good correlation in concentration 4-24 μg ml^{-1} with mean recovery 99.404±0.891. The optimum experimental parameters for the reactions have been studied and the reactions mechanisms are discussed. The validity of the described procedures was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The suggested procedures could be used for the determination of lisinopril in tablets. The procedures were rapid, simple and sensitive for quality control application.

1. INTRODUCTION

Lisinopril [N-[N-[(S)-1-Carboxy-3-phenylprolyl-]-L-lysyl]-L-proline dihydrate (Figure 1) is an angiotensin-converting enzyme (ACE) inhibitors used in the treatment of hypertension and heart failure[1].

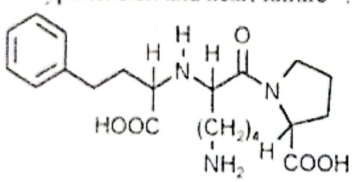

Figure 1: Chemical Structure of lisinopril

The drug and its tablets are official in USP (24)[2] where HPLC method is described for their quantitation. Various spectrophotometric methods have been reported for the determination of lisinopril using different reagents including 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole[3] 2,4-dinitrofluorobenz-ene[4] 1-fluoro-2,4-dinitrobenzene (Sanger reagent)[5] sodium hypochlorite and phenyl hydrazine[6] chloranil, dichlone and acetyl acetone with formaldehyde[7], second derivative spectrophotomet-ric[7,8] and spectrofluorimetric methods[7] were applied. HPLC[8], micellar electrokinetic chromatography[9], GLC[10], radioimmunoassay[11] and fluoroenzymic assay [12] have been reported. Lisinopril has a very low absorption in the UV region[13], as a result, interference from excipients may occur.

1-chlorobenzotriazole, as N-halogen compound that contain cyclic three nitrogen chain, has the capability to undergo certain chemical reactions which prove its usefulness in organic synthesis, thus, 1-CBT oxidizes alcohols to aldehydes and ketones, hydrazo to azo compounds and 1-amino-4,5-diphenyl triazole to diphenylacetylene, and 1-CBT is converted to benzotriazole hydrochloride[14].

1-CBT reagent was used for assay of some sulphur compounds as thiourea, allylthiourea, phenylthiourea, tolythiourea, thioacetamide, thiobenzamide, diethylthiocarbamate, ethylphenyldithiocarbamate, diisopropyldithiocarbamate, and methionine[15]. Recently, 1-CBT was used for determination some of phenothiazine derivative[16] and certain sulphur containing drugs, cefotaxime and cefuroxime[17].

On the other hand the reaction between ninhydrin or 1,2-naphthaquinone-4-sulphonate and α,β-amino acid, primary and secondary aliphatic amines has been applied extensively for the colorimetric determination of these compounds[18,19]

The aim of this work is to develop simple methods for the content uniformity analysis of lisinopril in dosage forms.

2. EXPERIMENTAL

2.1. Instrumental:

A Shimadzu 1601-UV-visible spectrophotometer was used.

2.2. Materials and reagents:

All materials and reagents used were of analytical grade and distilled water was used:

1. Lisinopril dihydrate pure drug and Zestril tablets (labelled to contain 5 mg lisinopril per tablet) were obtained from Sedico Co., 6th October City, Egypt, under licence from Zenica Ltd, England.
2. 1-Chlorobenzotriazole was prepared by method of Johnson et al [20] and recrystallized from dichloromethane, m.p. (105 - 106 °C). 0.1 % w/v prepared by dissolving 0.1 gm CBT in 10 ml DMF and diluting to 100 ml with distilled water..
3. Ninhydrin, Winlab, England, freshly prepared 2%, w/v solution in acetate buffer pH 5.
4. Potassium 1,2-naphthaquinone-4-sulphonate (NQS), freshly prepared 0.04%, w/v in distilled water.

2.3. Standard solution:

Aqueous and methanolic 0.4 $mgml^{-1}$ standard drug solutions were used.

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2.4. Sample preparation:

An accurately weighed amount equivalent to 10 mg lisinopril dihydrate was shaken with either 25 ml distilled water or methanol, used to suit each method.

2.5. Construction of calibration curves:

Method 1 (using CBT reagent)

Aliquots of standard aqueous lisinopril solution, in the concentration range cited in Table 1, were transferred into 10-ml volumetric flasks. 3 ml 0.1% CBT and 2 ml 0.1 M NaOH were added to each flask, allowed to stand for 25 min. at room temperature then completed to volume with distilled water. The absorbance was measured at 350 nm against a reagent blank.

Table (1): Optical characteristics and statistical data of the regression equations for determination of Lisinopril using the proposed methods

Parameters	Method 1 (using CBT)	Method 2 (using ninhydrin)	Method 3 (using NQS)
Linearity range (μgml^{-1})	8 -64	4 - 20	4 - 24
Molar absorptivity ($mol^{-1}l.cm^{-1}$)	5.7×10^3	1.55×10^4	2.10×10^4
Sandell's sensitivity (μgcm^{-1})	1.2×10^{-3}	3.5×10^{-3}	4.7×10^{-3}
Regression equation:			
Intercept (a)	0.0039	0.012	0.0842
Slope (b)	0.0126	0.0338	0.0376
Correlation coefficient (r)	0.9991	0.9994	0.9997

Method 2 (using Ninhydrin reagent)

Aliquots of standard methanolic lisinopril solution, within the concentration range presented in Table 1, were transferred into 10 - ml volumetric flasks. 1 ml 2% ninhydrin was added. The mixture was heated in a boiling water bath for 40 min., completed to volume with methanol and the absorbance was measured at 564 nm against a reagent blank.

Method 3 (using NQS reagent)

Into a series of 10 ml volumetric flasks, aliquot volumes of standard methanolic drug solution, within the concentration range stated in Table 1 were transferred. To each flask 3 ml 0.04 % NQS and 0.5 ml 0.01 M NaOH were added, allowed to stand for 25 min. at room temperature and completed to volume with distilled water. The produced colour was measured at 305 nm against a reagent blank.

2.6. Procedure for tablets:

An accurately weighed quantity of powdered tablets equivalent to 20 mg lisinopril was placed in a 50 ml volumetric flask 30 ml distilled water for method 1 or methanol for method 2 and 3 was added and the solution was shaken for 5 min. to dissolve the drug. The volume was made up to 50 ml with the same solvent and the solution was filtered and analyzed as above.

3. RESULTS AND DISCUSSION

The organic positive halogen compounds have been used as oxidizing agent for the oxidation of a variety of organic compounds. The oxidation reactions generally involve the abstraction of the hydrogen from -C-H, O-H, -N-H, or -S-H bonds. Though the reactions involving addition of oxygen have also been reported. These reactions have found extensive application in the determination of organic compounds. In this work, utilizing the oxidative properties of CBT was used for determination of lisinopril in pure form and in pharmaceutical preparations. Besides, the primary aliphatic amine of lisinopril was suggested to form condensation products with the carbonyl group of either NQS or ninhydrin and the produced colours was used for drug analysis at the specific λ_{max}. Figure 2.

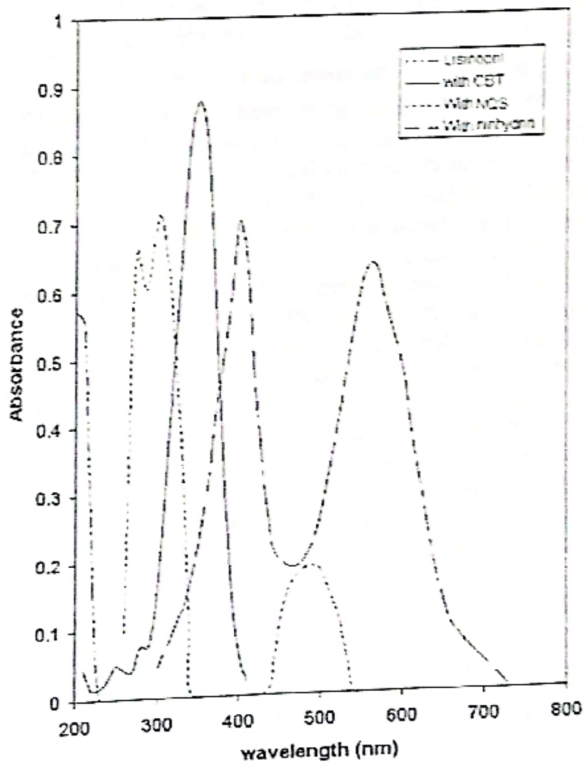


Figure 2: Absorption spectra of reaction product between CBT (10 $\mu g\ ml^{-1}$) method 1, ninhydrin (94 $\mu g\ ml^{-1}$) method 2 and NQS (10 $\mu g\ ml^{-1}$) method 3 and lisinopril (64, 16, and 18 μgml^{-1}), respectively.

3.1. Optimum reaction conditions:

The different experimental parameters affecting the colour development including reaction time, reagent concentration, solvent type and pH were studied.

The time required for complete reaction at room temperature was 25 min. for CBT and NQS methods, while heating for 40 min. in a boiling water bath was necessary to produce maximum absorbance in ninhydrin method.

The effect of pH was studied carefully, the reaction of lisinopril with CBT proceeds only in alkaline medium while in NQS method the absorbance was found to increase from pH 5 up to pH 10. So, 0.01 M NaOH was required to produce maximum colour intensity. The optimum concentration of NaOH was 2

ml 0.1 M and 0.5 ml 0.01 M aqueous solution for CBT and NQS methods, respectively. On the other hand, maximum absorbance was obtained at pH 5 in the method using ninhydrin reagent.

The amount of reagent necessary to obtain a linear graph for a drug concentration was studied. Maximum and reproducible colour intensity was produced when the amount of reagents mentioned in construction of calibration curves have been used. Higher concentrations of reagents did not affect the colour intensity.

For the effect of solvent, water was used to give maximum color intensity and stability in CBT method while in the other two methods water, ethanol, and methanol gave the same absorbance. So, water was used as diluting solvent for the three methods.

3.2. Chemistry of the coloured species

The composition of colored species was determined by Job's method of equimolar solutions. The concentration of aqueous lisinopril and CBT, NQS or ninhydrin were 2×10^{-3}, 1×10^{-3} and 5×10^{-3}, respectively. The plots indicate that the reactions were 1:4, 1:2 or 1:2, respectively, for the three colored species, Figure 3. For the reaction with NQS, the observation suggests that the primary amino group is involved in the colour development by condensation reaction while the secondary amino group by nucleophilic displacement of the sulfonic acid group of NQS (Scheme 1). For the reaction of lisinopril with ninhydrin, Scheme 2, suggests that the reaction product is due to oxidative deamination of the primary amino group followed by the colour reaction product –Ruhemenn's purple – with λ_{max} at 564.

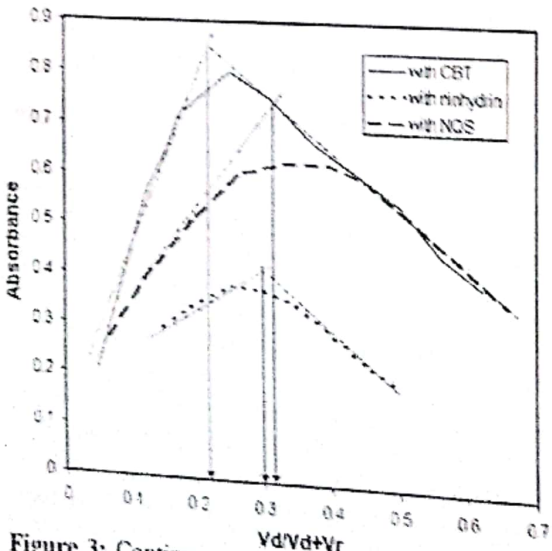
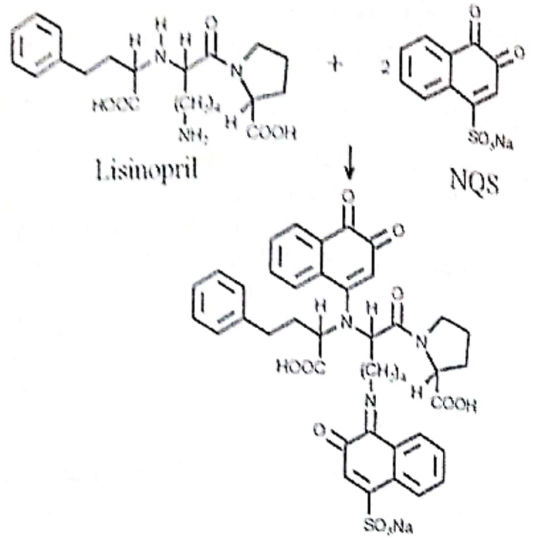
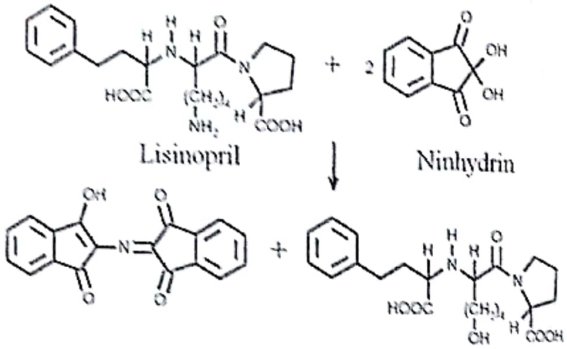


Figure 3: Continuous variation plots for the reaction product formed between lisinopril: CBT (2×10^{-3} M), lisinopril: NQS (1×10^{-3} M) and lisinopril: ninhydrin (5×10^{-3} M)



Scheme 1: The suggested reaction pathway between lisinopril and NQS



Scheme 2: The suggested reaction pathway between lisinopril and ninhydrin

3.2. Linearity, accuracy and precision:

The methods were tested for linearity, accuracy and precision. By using the above spectrophotometric procedures, linear regression equations were obtained. The regression plots showed a linear dependence of the absorbance over the Beer's law range given in Table 1. The table also shows the results of the statistical analysis of the experimental data, such as the slopes, the intercepts; the correlation coefficients obtained by the linear least squares treatment of the results

In order to determine the accuracy and precision of the methods solution containing three different concentration of lisinopril were prepared and analyzed in three replicates. The analytical results obtained from this investigation are summarized in Table 2. The mean relative standard deviation (RSD) can be considered to be very satisfactory.

The proposed methods were compared with a spectrophotometric reference method[15]. The results obtained showed that the calculated t- and F- values did not exceed the theoretical values (95% confidence limits for five degree of freedom), from which we can conclude that the proposed methods do not differ significantly from reference method. Table 3. The proposed methods were also, applied to commercial tablets contain lisinopril with mean recoveries 99.32±1.176, 100.18±0.761 and 100.14±0.844, applying methods 1, 2 and 3, respectively. The results show that there is no interference from any excipients.

Table (2): Evalution of the accuracy and precision of the proposed methods

Method 1 (using CBT reagent)			Method 2 (using ninhydrin reagent)			Method 3 (using NQS reagent)		
Taken μgml^{-1}	Found μgml^{-1}	Recovery %	Taken μgml^{-1}	Found μgml^{-1}	Recovery %	Taken μgml^{-1}	Found μgml^{-1}	Recovery %
24	23.95	99.79	4	4.03	100.75	8	8.02	100.25
	23.65	98.54		3.98	99.50		7.94	99.25
	24.05	100.20						
40	40.08	100.20	12	11.80	98.33	16	15.94	99.62
	40.32	100.80		11.71	97.58		15.72	98.25
	39.13	97.82		11.80	98.33			
56	55.00	98.21	20	19.94	99.70	24	24.14	100.58
	55.88	99.78		19.89	99.45		23.59	98.29
	55.00	98.21					23.90	99.58
Mean		99.28			99.09			99.40
N		9			7			7
V		1.184			1.143			0.795
SD		1.088			1.069			0.891
RSD		1.095			1.078			0.896

Table (3): Determination of lisinopril using the proposed methods compared with reference method[13].

	Proposed methods			Reference method[a] [13]
	Method 1 *(using CBT)*	Method 2 *(using ninhydrin)*	Method 3 *(using NQS)*	
Mean ± SD	99.28± 1.088	99.09± 1.069	99.40± 0.891	99.94 ± 0.967
N	9	7	7	5
t:	1.127 (2.179)	1.409(2.228)	0.999 (2.228)	
F:	1.264 (3.84)	1.221(4.53)	1.177 (4.53)	

[a] spectrophotometer method at λ_{max}= 267 nm

Values in parentheses are the tabulated values of t- and F- at p=0.05

CONCLUSION

The proposed methods provide simple, accurate and reproducible methods of determination lisinopril in pure form and in tablets. The three methods overcome the problem of low absorptivity of the drug in UV region.

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التقدير الطيفي لليزينوبريل في المستحضرات الصيدلية

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تم تعيين الليزينوبريل في الأقراص باستخدام ثلاث طرق تحليل طيفي بسيطة وحساسة. تتضمن الطريقة الأولي أكسدة الدواء باستخدام ١-كلوروبنزوترايازول في وسط قلوي وقياس الامتصاص الضوئي عند طول موجي يساوي ٣٥٠ نانومتر. بينما تعتمد الطريقة الثانية علي تفاعل مجموعة الأمين الموجودة في مركب الليزينوبريل مع كاشف النهيدرين وقياس ناتج التفاعل عند طول موجي ٥٦٤ نانومتر. بينما تتضمن الثالثة تفاعل اقتران بين الليزينوبريل و ٢،١- نافثوكينون-٤- سلفونات ليكون مركب ملون ذا طول موجي ٣٠٥ نانومتر. ولقد تمت دراسة العوامل المؤثرة علي التفاعلات وتم التأكد من فاعلية الطريقة باستخدام التحليل الإحصائي وبذلك تم إثبات دقة الطريقة وصلاحيتها لتقدير الدواء في مستحضراته حيث أن الطريقة سريعة وبسيطة وحساسة ويمكن استخدامها في قياس الجودة.