ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
19 (1999) 819–827

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms

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Received 2 January 1998; received in revised form 30 March 1998; accepted 2 April 1998

Abstract

Simple and sensitive methods are described for the assay of lisinopril in tablets. The first method (A) is based on the reaction of the drug with chloranil in aqueous solution of pH 9.5 to give yellow colour measured at 346 nm. The second method (B) is based upon the interaction of lisinopril with dichlone resulting in the formation of an intense purple colour measured at 580 nm. The third method (C) depends on the reaction of the drug with acetylacetone and formaldehyde to form a coloured condensation product measured at 356 nm and also has a strong fluorescence at 475 nm (λ_{ex} 410 nm). This method is extended to determine lisinopril in binary mixtures with hydrochlorothiazide. The last method (D) depends on measuring the first and second derivative spectra of lisinopril. Moreover, the derivative method is used as stability-indicating method where lisinopril can be determined in presence of its degradation products. The proposed methods proved to be suitable for a rapid quality control of commercial dosage forms. The results obtained were precise and accurate.

Keywords: Spectrophotometry; Spectrofluorometry; Lisinopril; Degradation products; Hydrochlorothiazide; Tablets

1. Introduction

Lisinopril (LN) is an effective angiotensin converting enzyme inhibitor used as antihypertensive [1]. The literature presents few methods for its determination either in biological fluids or in pharmaceutical preparations. These include HPLC [2], GLC [3], radioimmuno-assay [4] and spectro-photometry [5]. A HPLC method [6] is reported for the determination of LN and hydrochlorothiazide (HCT) in pharmaceutical preparations.

However, these methods require multistep extraction procedures and selective detectors. therefore, it was felt useful to develop spectrophotometric and fluorometric methods for its determination. The problem in the assay of LN is the precise, specific and easy measurement of this potent drug in dosage forms specially it only possesses a very low absorption in the UV region [7]. This weak absorption means that a conventional UV spectrophotometric assay of LN is susceptible to interference from excipients.

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0731-7085/99/$ - see front matter
PII S0731-7085(98)00110-1

Therefore, the aim of this work is to develop simple methods for the content uniformity analysis of LN in dosage forms. Also, to develop a stability-indicating method for the assay of LN in presence of its acid induced degradation products.

2. Experimental

2.1. Apparatus

A Perkin–Elmer model 550 S UV-VIS spectrophotometer, with 1-cm quartz cells and a Hitachi model 561 recorder were used.

A Perkin–Elmer model 650-10 S spectrofluorometer equipped with 1-cm quartz cells and a Perkin–Elmer model 56 recorder were used. Schott–Gerate pH meter model CG 710 was used.

2.2. Materials and reagents

All materials and reagents used were of analytical reagent grade. Lisinopril dihydrate was kindly provided by Sedico, Egypt. Zestril tablets (ICI, UK), containing 20 mg lisinopril anhydrous per tablet, and Acer–Comp–mite tablets (Zeneca) containing 20 mg lisinopril and 12.5 mg hydrochlorothiazide per tablet were purchased from the local market. Chloranil (BDH, Poole, UK) was prepared in ethanol (1 mM). Dichlone (Aldrich) was prepared to contain (15 mM) in acetone. The reagent for method (C) was prepared by mixing 10 ml of walpole acetate buffer solution pH 3.6 [8] with 2.1 ml of acetylacetone (BDH, Poole, UK) and 5 ml of formaldehyde (BDH, Poole, UK) and the mixture was completed to 25 ml with distilled water. Borax buffer pH 9.5 and walpole acetate buffer pH 3.6 were used.

2.3. Reference drug solutions

1. Stock solution of lisinopril dihydrate containing 1 mg ml^{-1} was prepared in methanol. Further dilutions were made to suit each method.
2. Stock solution of hydrochlorothiazide containing 1 mg ml^{-1} was prepared in methanol.

2.4. Sample preparations

1. An accurately weighed amount of powdered tablets equivalent to 100 mg of lisinopril dihydrate was transferred into a beaker with 50 ml methanol and stirred for 45 min. The extract was filtered into a 100-ml volumetric flask and completed to volume with methanol. Further dilutions of the filtrate were made to suit each method.
2. An accurately weighed quantity of the powdered tablets equivalent to 20 mg LN dihydrate and 12.5 mg HCT was transferred into 25 ml volumetric flask and extracted with methanol by shaking for 30 min. The volume was completed with methanol and filtered.

Table 1
Assay parameters for the analysis of LN by the proposed methods

Method	Conc. range (μg ml^{-1})	λ (nm)	Linear regression			ϵ (l mol^{-1} cm^{-1})	C.V. %
			Intercept (a)	Slope (b)	Corr. Coeff. (r)		
(A)	4–20	A_{346}	0.0267	4.69×10^{-2}	0.9999	2.07×10^{4}	0.81
(B)	40–120	A_{580}	0.0117	5.97×10^{-3}	0.9998	2.6×10^{3}	0.89
(C)	6–42	A_{356}	−0.0174	0.0218	0.9997	9.62×10^{3}	0.91
	0.03–0.27	F_{475} (λ_{ex} 410)	2.503	283.69	1.000	—	0.14
(D)	40–240	$D_{1\,256}$	−3.333	0.3803	0.9999	—	0.59
		$D_{2\,266}$	−1.366	0.3621	0.9999	—	0.64

ϵ, Apparent molar absorptivity.
C.V. %, Intraday coefficient of variation ($n = 5$).

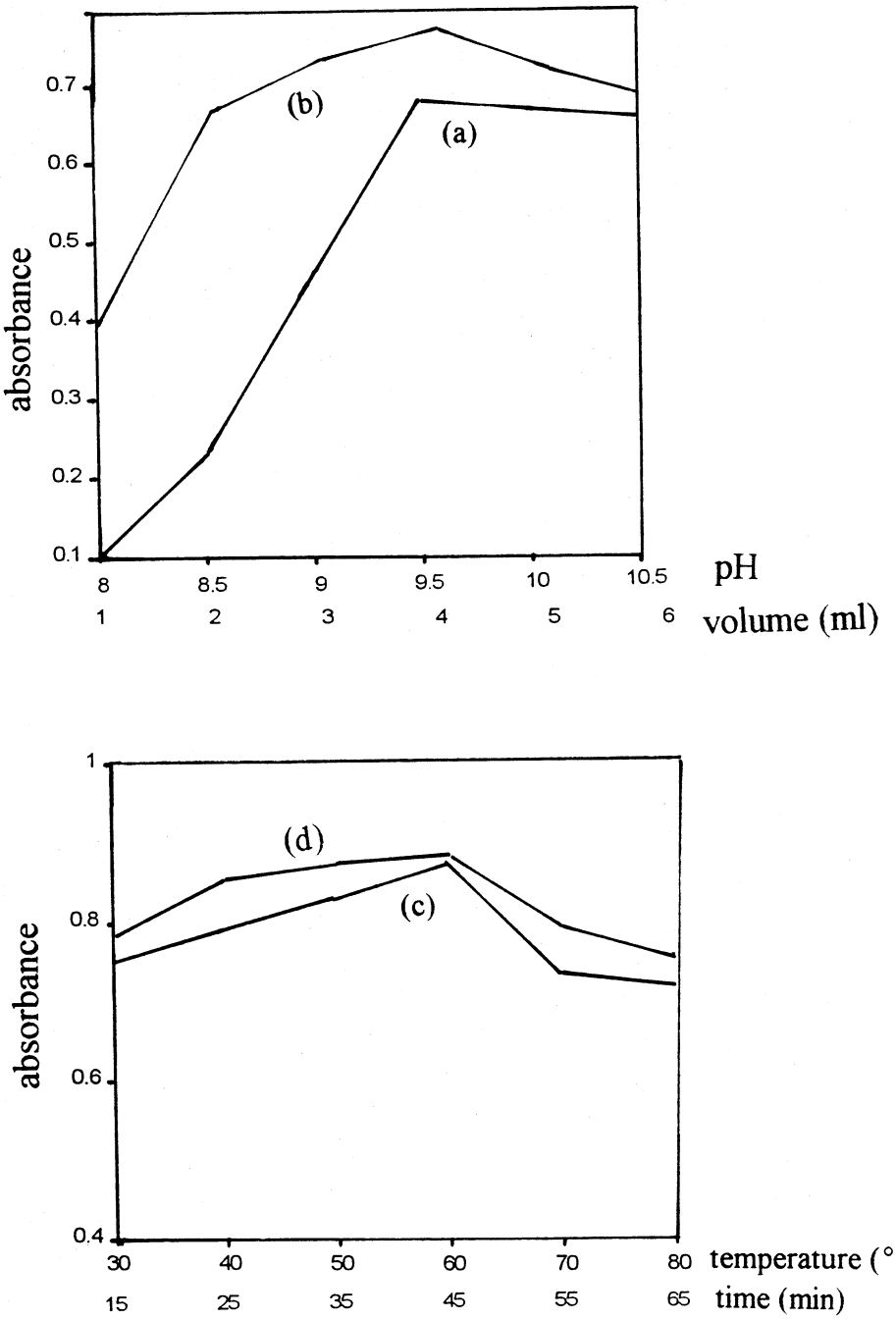

Fig. 1. Effect of pH (a), reagent concentration (b), temperature (c) and heating time (d) on the reaction of 18 μg ml^{-1} of LN with CH.

2.5. Construction of calibration curves

2.5.1. Method (A)

Aliquots of standard LN solution, in the concentration range cited in Table 1, were transferred into 10-ml volumetric flasks. To each flask, 2 ml of buffer pH 9.5 and 4 ml of chloranil solution were added, mixed and allowed to stand at 60°C for 45 min. After cooling, the solution was diluted to volume with distilled water. The absorbance of the resultant colour was measured at 346 nm against a reagent blank.

2.5.2. Method (B)

Portions of standard LN solution in dimethyl sulphoxide within the concentration range stated in Table 1, were transferred into 10-ml volumetric flasks. To each flask, 2 ml of dichlone in acetone were added, mixed well and allowed to stand at room temperature for 10 min. The solution was diluted to volume with acetone and the absorbance of the resultant colour was measured at 580 nm against a reagent blank.

2.5.3. Method (C)

Aliquots of standard LN solution, within the concentration range presented in Table 1, were transferred into 10-ml volumetric flasks. To each flask, 1 ml of the reagent was added, mixed well and heated in a boiling water bath for 10 min. The volume was completed with water and the absorbance of the solution was measured at 356 nm against blank, while the fluorescence was measured at 475 nm ($\lambda_{ex} = 410$ nm).

2.5.4. Method (D)

Portions of standard LN solution, in the concentration range cited in Table 1, were completed to 10 ml with methanol and the peak amplitude at 256 and 266 nm were measured for D_1 and D_2 spectra, respectively.

Five aliquots of standard HCT solution, within the concentration range 0.4–1.0 mg/100 ml, were completed to 100 ml with 0.1 N HCl and the D_1 peak amplitude at 322 nm was measured for each solution.

3. Preparation of acid-induced degradation product

A quantity of 40 mg of lisinopril dihydrate were transferred into 50-ml volumetric flask with least amount of water. Then 25 ml conc. sulphuric acid

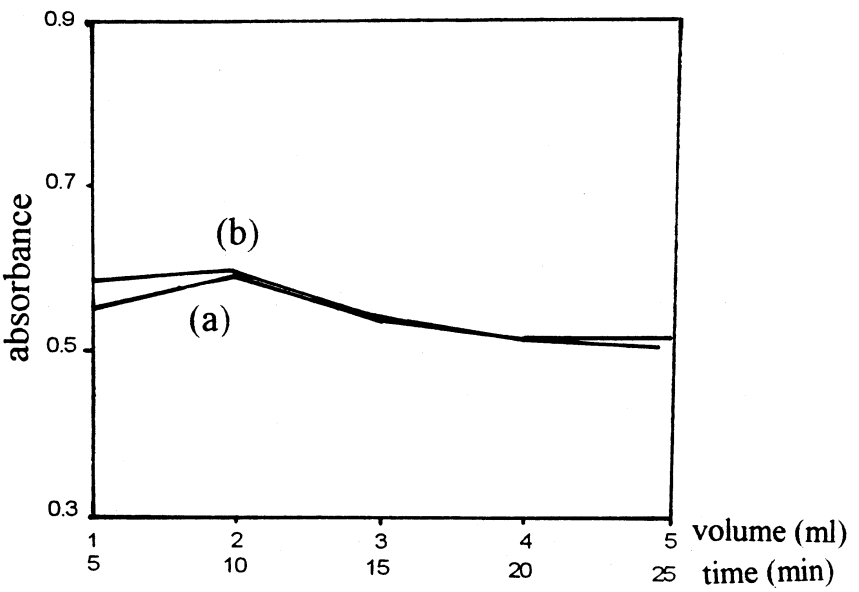

Fig. 2. Effect of reagent concentration (a) and time (b) on the reaction of 100 μg ml^{-1} of LN with DI.

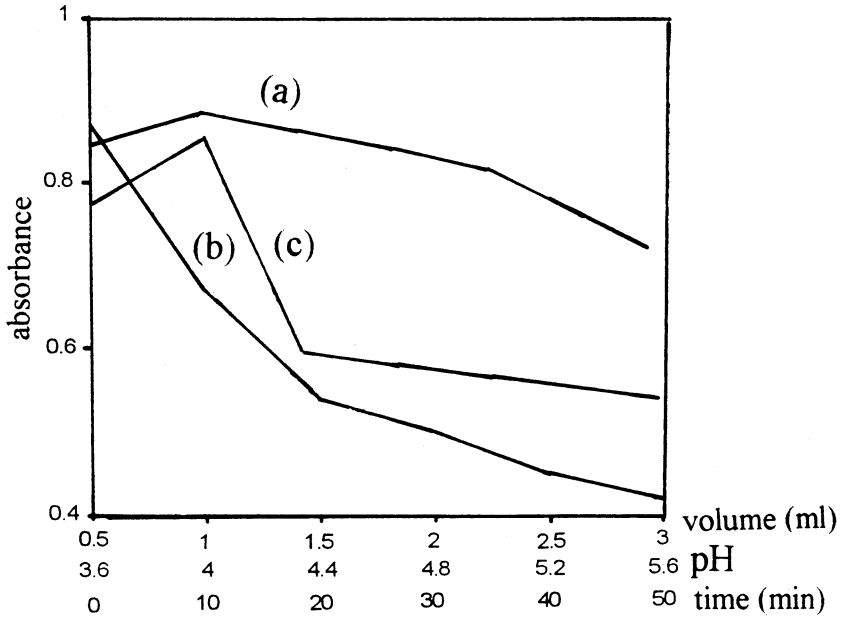

Fig. 3. Effect of reagent concentration (a), pH (b) and heating time (c) on the reaction of 40 μg ml^{-1} of LN with Hantzsch reagent.

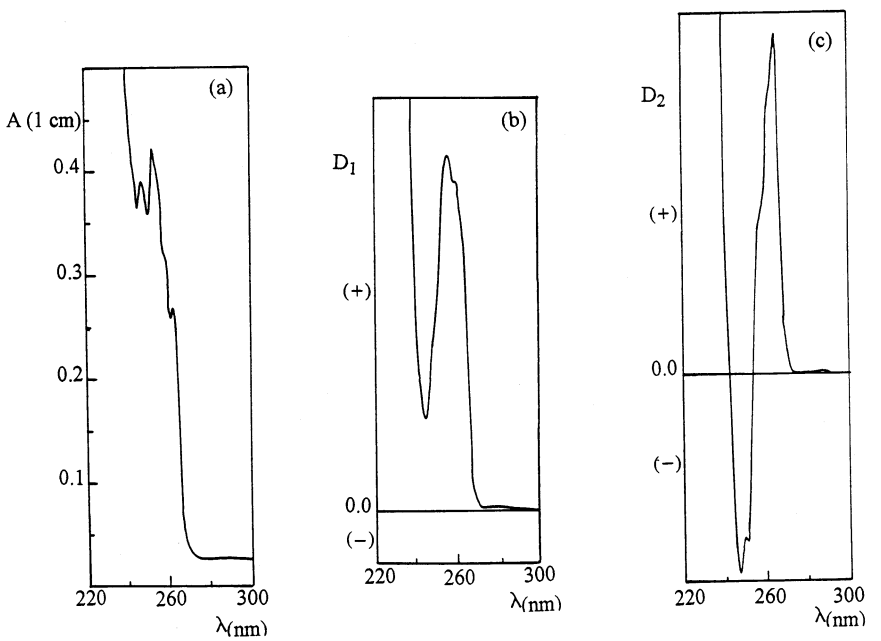

Fig. 4. Absorption (a); first derivative (b) and second derivative (c) spectra of Lisinopril dihydrate (800, 240, and 240 μg ml^{-1}, respectively) in methanol versus blank.

were added and the solution was heated in a boiling water bath for 1 h. The solution was cooled and diluted to volume with distilled water.

Table 2
Assay results for the determination of LN in its tablets by the proposed methods

Tablet preparation	Label claim (mg tablet^{-1})	% Found ± S.D. ($n = 5$)				
		A_{max} method	Proposed methods			
			A	B	C	D
Zestril tablets	20	124.98 ± 5.44	98.9 ± 0.79	98.4 ± 0.34	98.71 ± 0.62	100 ± 0.73	99.07 ± 0.51	99.2 ± 0.73
t			0.624	2.221	1.144	1.733		
F			1.171	4.61	1.386	1.00		

Theoretical values for t- and F-tests at $p = 0.05$ are 2.31 and 6.39, respectively.

4. Results and discussion

4.1. Method (A)

The reaction of chloranil with nitrogenated drugs results in the formation of coloured products [9]. LN reacts with chloranil in aqueous medium to form a yellow product which exhibits absorption maximum at 346 nm. The reaction conditions were studied as a function of the volume of the reagent, reaction time, reaction temperature and the volume and pH of the buffer (Fig. 1). The described procedure gives maximum stability and sensitivity as the calculated molar absorptivity, ϵ, is 2.07×10^4.

4.2. Method (B)

The reaction of dichlone with basic nitrogenated drugs has been described as charge transfer complex formation [10]. Being an amine, LN reacts as n-electron donor with dichlone, a pi-acceptor, to give a blue coloured product with maximum absorption at 580 nm. The different parameters affecting the colour development were extensively studied so as to give the best sensitivity and stability, a minimum blank reading and adherence to Beer's law (Fig. 2).

4.3. Method (C)

LN, as a primary amine, undergoes Hantzsch condensation reaction, it reacts with acetylacetone and formaldehyde to form an intense coloured condensation product with maximum absorption at 356 nm [11]. Moreover, the condensation product exhibits strong fluorescence at 475 nm ($\lambda_{ex} = 410$ nm) which permits the development of a very sensitive method of assay for LN in its tablets. A study of the effect of the concentration of reagents, pH of the buffer, and time of the reaction (Fig. 3), led to the described procedure.

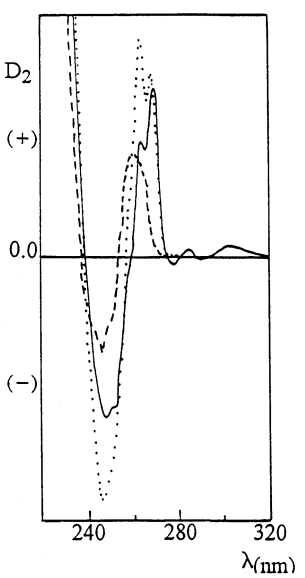

Fig. 5. Second derivative spectra of Lisinopril (80 μg ml^{-1} (– – –); degraded product (80 μg ml^{-1}) (——) and their mixture (· · ·).

Table 3
Assay results for the analysis of LN in synthetic mixtures with degradation products by D_2-method

No	Added Conc. (μg ml^{-1})		Ratio LN:Deg. LN	Found LN (μg ml^{-1})	% Recovery
	LN	Deg. LN			
1	80	8	1:0.1	80.4	100.50
2	80	16	1:0.2	80.4	100.50
3	80	40	1:0.5	80.4	100.50
4	80	60	1:0.75	81.4	101.78
5	80	80	1:1	80.4	100.50
Mean ± S.D.					100.72 ± 0.52

4.4. Method (D)

The zero order spectrum of LN in methanol has no characteristic absorption bands (Fig. 4), in addition the drug is weakly absorbing in the UV region (A 1%/1 cm 4.5 at 258 nm). Hence conventional spectrophotometric method cannot be applied for its determination due to interference from formulation matrix. On the other hand, the derivative technique was used to increase the characteristic information about the shape of the absorption curve. Therefore, the first (D_1) and second (D_2) derivative spectra of LN exhibit characteristic peaks at 256 and 266 nm, respectively (Fig. 4). Thus, the absolute D_1 and D_2 peak amplitude measurements at these wavelengths were selected for its determination.

Under the described experimental conditions of the above mentioned methods, plots of absorbance, relative fluorescence, D_1 and D_2 values versus concentrations within the range stated in Table 1 show linear relationships. The regression analysis of these plots using the method of least squares were made for the slope (b), intercept (a) and correlation coefficient (r) values (Table 1). The linearity of the calibration graphs were validated by the high values of correlation coefficients of the regression equations and by the intercept values, which were close to zero. The high sensitivity of the proposed methods were indicated by the values of the molar absorptivities of the developed colour products (Table 1). The fluorimetric method is about 100 times more sensitive than the other methods. The intraday coefficients of variation (CV%) calculated for separate determinations at different concentration levels of each method did not exceed 1% (Table 1). This indicates good precision and reproducibility of the developed methods.

The commercial tablets of LN in single dosage form were assayed using the proposed methods. The extent of interference in tablets is evident from the results obtained from the conventional A_{max} method. The assay results for tablets, obtained by the described methods are in good agreement with the declared amount (Table 2) indicating high accuracy of the methods. Also the results obtained by the proposed methods are in accord with each other (Table 2).

Furthermore, the proposed derivative spectrophotometric method is extended to be used as stability-indicating method for the determination of intact LN in presence of its acid-induced degradation products. It has been reported [7] than, LN decomposition proceeds rapidly in acidic media with the major decomposition product being the diketopiperazine. In neutral and basic media, the decomposition rate is minimal. The use of A_{max} method for the determination of the intact drug will give unacceptable results due to the spectral overlapping of the degraded products. On the other hand, the D_2 spectra show that LN possesses a maximum D_2 value at 259.5 nm, while that of its degradation products equals to zero at the same wavelength (Fig. 5). Therefore, the absolute D_2 value at 259.5 nm can be used to quantitate the intact drug without any interference from degradation products. The graph obtained by plotting D_2 values against concentration of LN shows a linear relationship over a concentration

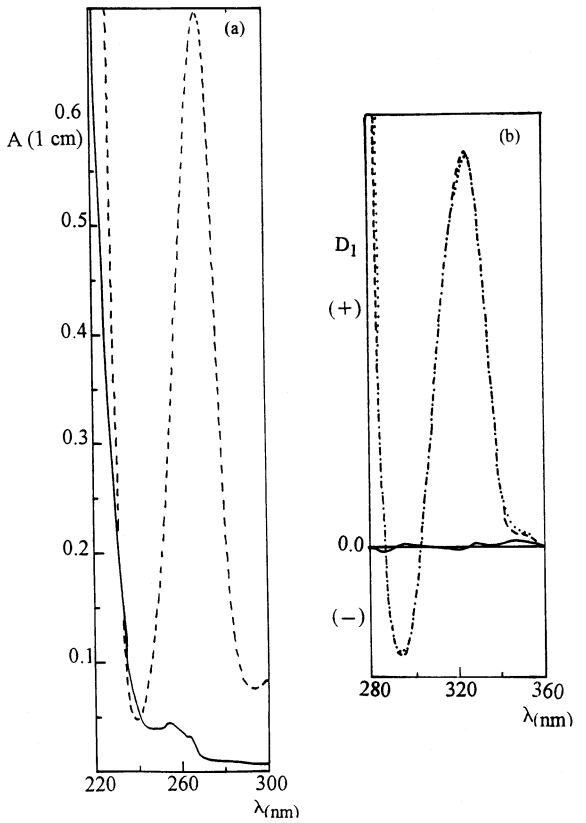

Fig. 6. (a) Absorption spectra of Lisinopril (80 μg ml^{-1}) (——) and hydro-chlorothiazide (10 μg ml^{-1}) (· · ·) in methanol vs. Blank. (b) First derivative spectra of Lisinopril (12.8 μg ml^{-1}) (——), hydrochlorothiazide (8 μg ml^{-1}) (– – –) and their synthetic mixture (· · ·) in methanolic HCl.

Table 4
Assay results for the analysis of LN in synthetic mixtures containing LN and HCT by Hantzsch reaction

No.	Conc. (μg ml^{-1})			
	Added		Found LN	%recovery
	LN	HCT		
1	24	7.5	24.27	101.15
2	24	10	24.14	100.58
3	24	15	24.32	101.34
4	24	22.5	24.4	101.92
5	24	30	24.36	101.54
Mean ± S.D.				101.3 ± 0.50

range 4–16 mg/100 ml according to the following equation

$$D_2 = 1.7321 + 4.8839C \quad (r = 0.9995)$$

where C is the concentration of LN in mg/100 ml and D_2 is its peak height in mm at 259.5 nm. In order to prove the validity of the method, five synthetic mixtures containing 8 mg/100 ml intact LN and different amount of the degradation products, in the concentration range of 0.8–8 mg/100 ml, were prepared. The concentration of the intact LN in these mixtures was calculated using the D_2 method. The results obtained are satisfactory (Table 3). These results indicate that the minimum level at which the LN can be determined with acceptable accuracy and precision is at 50% degradation, this means that when the ratio of intact LN to deg. LN is 1:1.

The combination of LN and HCT as a tablet preparation has been recently introduced in the market for treatment of hypertension. The literature reveals only one HPLC method [6] for the analysis of this combination. But till now there is no spectrophotometric method has been reported for its analysis. The problem which has arisen during the analysis of this mixture is that LN possesses only a very low absorption in the UV region while HCT exhibits very great absorption in the same region (A 1%/1 cm 644 at 272 nm) [12] (Fig. 6). This weak absorption means that a conventional UV method or derivative method for the assay of LN is susceptible to interference from HCT. The derivative and derivative differential techniques [13] cannot cope with the level of interference due to the great spectral overlapping of HCT. Also the derivative compensation technique [14] fails to determine LN due to its very low contribution in the gross absorption curve of the mixture. Therefore, depending on the fact that LN contains a primary amino group, its reaction with acetylacetone and formaldehyde was utilized to develop a spectrophotometric method for its analysis in combination with HCT where the latter did not undergo the same reaction. On the other hand, HCT was determined in this combination by measuring the D_1 amplitude at 326 nm where LN has no contribution (Fig. 6), the graph obtained by plotting the D_1 value of HCT against

concentration shows a linear relationship within the range 0.4–1.0 mg/100 ml according to the following equation:

$$D_1 = -1.5071 + 59.214C \quad (r = 0.9997)$$

where C is the concentration in mg/100 ml and D_1 is the peak height in mm at 326 nm.

To prove the validity and the applicability of the proposed method, five synthetic mixtures were prepared in different proportions of LN and HCT (Table 4), and the concentration of LN was determined. The results obtained (Table 4) were both precise and accurate, the mean percentage recoveries obtained for the determination of HCT in these mixtures by the proposed derivative method was 98.39 ± 0.47.

The commercial tablets containing LN and HCT were assayed by the proposed method, the percentage recoveries obtained for the contents of LN and HCT were 99.59 and 98.95 respectively. The results obtained show the high reliability and reproducibility of the method.

The developed methods are rapid, accurate and sensitive that overcome the problem of low absorptivity of LN in UV region and can be used for the routine analysis of LN in its single or multicomponent pharmaceutical preparations.

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