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Spectrophotometric, septrofluorimetric and LC determination of lisinopril

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Abstract

Three methods are described for the determination of lisinopril in the pharmaceutical tablets. The spectrophotometric method depends on the reaction of the lisinopril with sodium hypochlorite and phenyl hydrazine to form a condensation product measured at 362 nm. The spectrophotometric method was extended to develop a stability indicating method. The spectrofluorimetric method depends on reaction of the lisinopril with *o*-phthalaldehyde in the presence of 2-mercaptoethanol in borate buffer pH 9.5. The fluorescence of the reaction product was measured upon excitation at a maximum of 340 nm with emission wavelength at 455 nm. The HPLC method depends on using Hypersil silica column with a mobile phase consisting of methanol–water–triethylamine (50:50:0.1 v/v) and the pH was adjusted to 2.6 with 0.1 N perchloric acid. Quantitation was achieved with UV detection at 210 nm based on peak area.

Keywords: HPLC; Lisinopril; Spectrofluorometry; Spectrophotometry

1. Introduction

Lisinopril, (S)-1-[N^2-(1-Carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate, is an angiotensin converting enzyme inhibitor that is used in the treatment of hypertension and heart failure [1]. The official methods for the determination of lisinopril are potentiometric acid–base titration [2] and HPLC [3] using octylsilane column at 50°C and phosphate solution – acetonitrile (96:4 v/v) as mobile phase. Various spectrophotometric methods have been reported for the determination of lisinopril in pharmaceutical tablets using different reagents including chloranil, dichlone and acetylacetone with formaldhyde [4]. First, second derivative spectrophotometric [4,5] and spectrofluorometric methods [4] were applied. The chromatographic techniques of analyses, HPLC using Hypersil ODS column [5], micellar elec-

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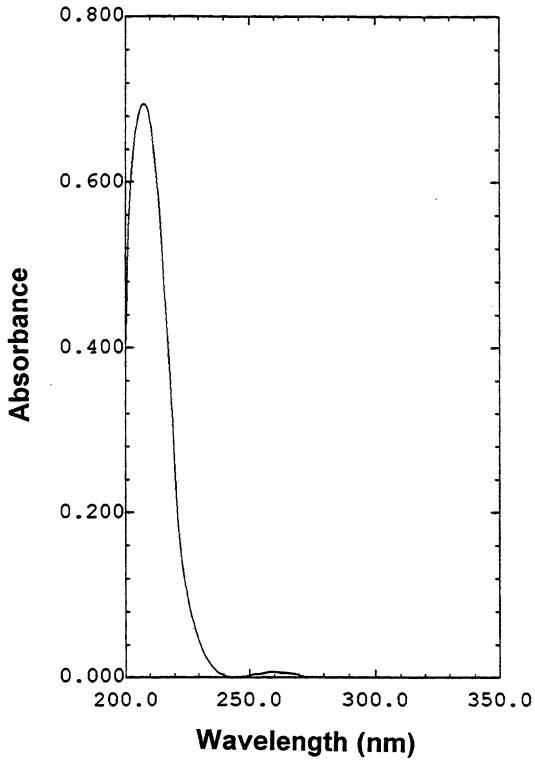


Fig. 1. UV absorption spectrum of 20 μg ml^{-1} of lisinopril in 0.1 M hydrochloric acid.

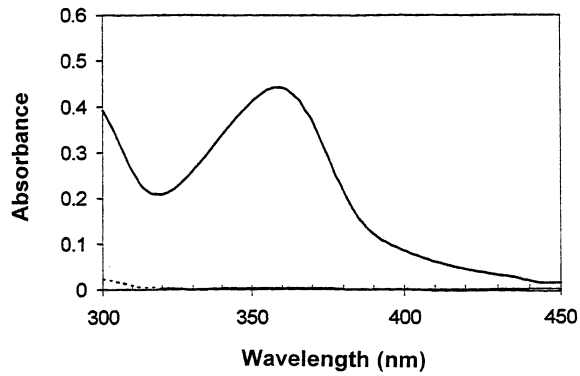
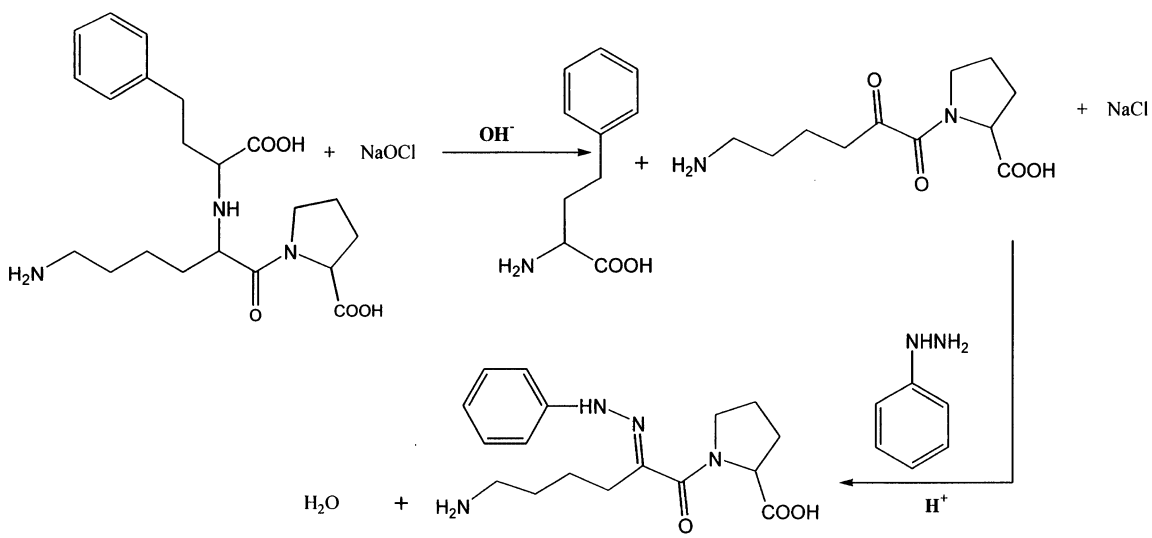


Fig. 2. Absorption spectra of 90 μg ml^{-1} of both lisinopril (—)and its degradation product, diketopiperazine, (-------) after condensation reaction with phenylhydrazine, measured against reagent blank.

trokinetic chromatography [6] and gas liquid chromatography [7] have been employed. Capillary electrophoresis was applied to the determination of lisinopril in pharmaceutical tablets [8]. The literature presents few mehods for determination of lisinopril in biological fluids. These include HPLC using μ Bondapak C_{18} column at 45°C [9], fluoroimmunoassay [10], radioimmunoassay [11] and fluoroenzymatic assay [12]. Many spectrophotometric methods have been applied for the simultaneous determination of lisinopril and hydrochlorothiazide in binary mixture including



Scheme 1. Proposed reaction between lisinopril and sodium hypochlorite followed by condensation with phenylhydrazine.

measurement of the absorbance at 205 and 225 nm [13]. Vierordt method, first derivative spectrophotometry, ratio spectra first derivative spectrophotometry [14] and combination of spectrophotometric method using acetylacetone and formaldehyde with first derivative spectrophotometric method [4]. The literature reveals one HPLC method for the analyses of this binary mixture using Hypersil ODS column [15].

Lisinopril possesses a very low absorption in the UV region [16]; as a consequence, poor sensitivity can be achieved by conventional UV spectrophotometric method. Moreover, reversed phase high performance liquid chromatography of this drug may show peak splitting owing to slow *cis–trans* isomerization, caused by hindered rotation around the *N*-substituted peptide bond [5]. Therefore, the aim of this work is to develop sensitive spectrophotometric, spectrofluorometric methods, and a HPLC method able to avoid peak splitting. Also, to develop a stability-indicating method for the assay of lisinopril in presence of its acid induced degradation products. The official HPLC method [3] and other HPLC method [9] carry out the determination of lisinopril at elevated column temperature, while the proposed HPLC method has advantage of using the column at ambient temperature. The three proposed methods are more simple than the other published method. They are suitable for routine determination of lisinopril in pharmaceutical products.

Table 1
Determination of lisinopril (I) in presence of its acid induced degradation product, diketopiperazine, (II) in laboratory prepared mixtures using spectrophotometric method

Mixture number	Mixture composition ($\mu g\ ml^{-1}$)		% Recovery of I
	I	II	
1	100	2	98.8
2	120	3.5	101.6
3	140	5	99.5
4	160	7	101.6
5	180	9	99.5
6	200	15	100.1
7	200	20	98.8
8	200	40	99.6
Mean ± S.D.			99.94 ± 1.11

2. Experimental

2.1. Instrumentation

A double beam Shimadzu (Japan) UV-visible spectrophotometer, model UV-1601 PC, connected to IBM compatible computer and a HP600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. The absorption of test and reference solutions was recorded in 1-Cm quartz cells.

Shimadzu spectrofluorophotometer, model RF-540 was used. The fluorescence spectra of test and reference solutions were recorded in 1-Cm quartz cells.

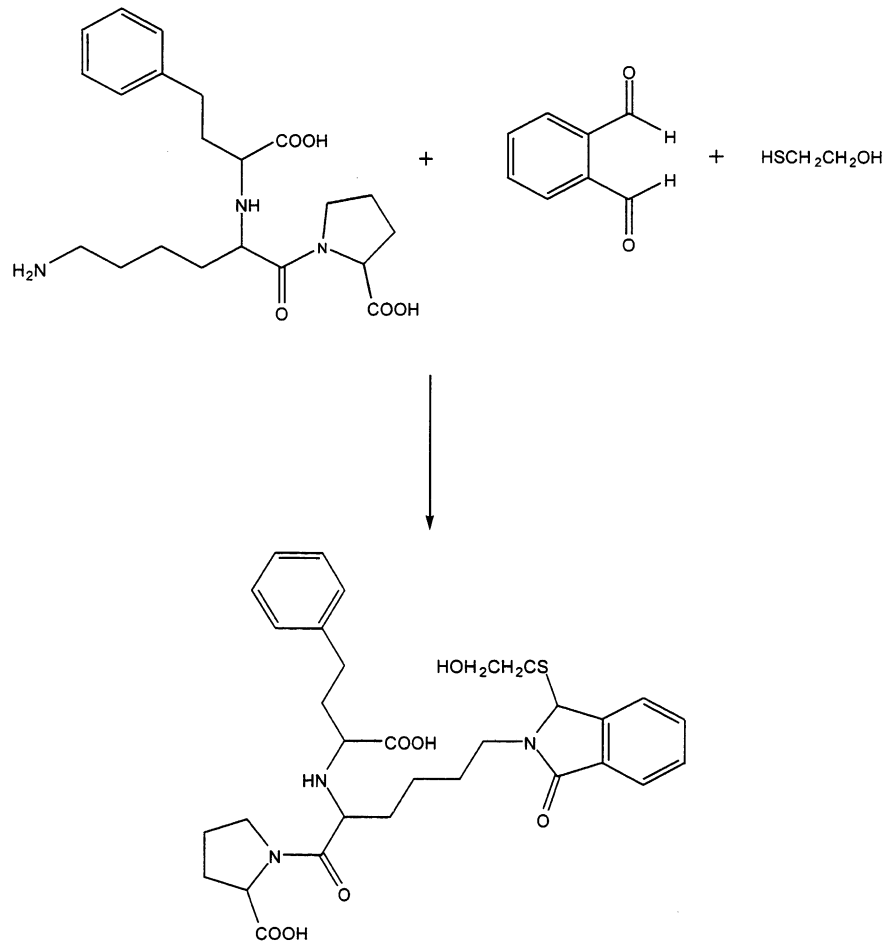
The HPLC (Perkin–Elmer, Norwalk, CT, USA) instrument was equipped with a model 410 LC pump, Rheodyne 7125 injector with a 20-μl loop and a LC-235 diode array detector (Perkin–Elmer). Separation and quantitation were made on a 250 × 4.6 mm (i.d.) ILS® Hypersil Silica (5 μm particle size). The detector was set at γ 210 nm. Data acquisition was performed on a model 1022 PE Nelson (Perkin–Elmer).

2.2. Materials and reagents

Pharmaceutical grade of lisinopril dihydrate was kindly supplied by Sedico, 6th October City, Egypt, and certified to contain 100.0%. The water for HPLC was prepared by double glass distillation and filtration through 0.45-μm membrane filter. The methanol used was HPLC grade (BDH, Poole, UK). Other reagents were of analytical grade.

Borate buffer (0.05 M) pH 9.5 was prepared by dissolving 19.07 gm borax in sufficient distilled water to produce 1000 ml and the pH was adjusted to 9.5 using 0.1 M sodium hydroxide.

O-phthalaldhyde buffered reagent was prepared just before use according to Roth's method [17] by mixing 1.4 ml of *o*-phthaladehyde solution (10 mg ml^{-1} in methanol) with 90 ml of borate buffer



Scheme 2. Proposed reaction between lisinopril and *O*-phthalaldehyde in presence of 2-mercaptoethanol.

pH 9.5, then 1.4 ml of 2-mercaptoethanol (5 μl ml^{-1} in methanol) was added.

Phenylhydrazine HCl solution (1 mg ml^{-1}) was prepared in 50% sulphuric acid.

0.2 N sodium hypochlorite solution was prepared by diluting 5% aqueous solution of sodium hypochlorite and adjusting normality of the solution iodometrically.

2% sodium bisulphite solution was freshly prepared.

The commercial zestril tablets used were manufactured by Sedico, 6th October City, Egypt, under license from Zenica Ltd, England. Each tablet contain lisinopril dihydrate equivalent to 5 mg (Batch No. 2997106), 10 mg (Batch No. 2995107) and 20 mg (Batch No. 1096101) anhydrous lisinopril, in addition to tablet excipients consisting of maize starch, calcium hydrogen phosphate, magnesium stearate, mannitol and red ferric oxide.

2.3. HPLC conditions

The mobile phase was prepared by mixing methanol, water, and triethylamine in a ratio of 50:50:0.1 v/v and the pH was adjusted to 2.6 using 0.1 N perchloric acid.

The mobile phase was filtered using a 0.45 μm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The samples

were also filtered using 0.45 μm disposable filters. The flow rate was 1 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Preparation of the acid-induced degradation product

A quantity of 100 mg of lisinopril dihydrate was transferred into a conical flask. Then 100 ml of 50% sulphuric acid was added. The solution was refluxed for 2 h at 100°C. Subsequently, the solution was cooled and neutralized with calcium carbonate powder. The neutralized solution was filtered and evaporated to dryness under reduced pressure. The residue was tested for complete degradation using the TLC system with aluminum sheets silica gel $60F_{254}$ (Merck) and chloroform: ethyl acetate: acetic acid (10:3:2 v/v) as a mobile phase. A single spot at $R_f = 0.17$ was observed. While no spot was observed at $R_f = 0.31$ corresponding for lisinopril dihydrate.

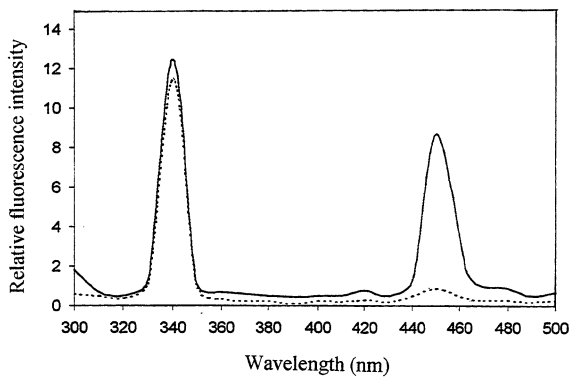


Fig. 3. Spectral characteristics of the excitation and emission spectra of 110 ng ml^{-1} of lisinopril after reaction with *O*-phthalaldehyde buffered reagent (—) and reagent blank (-------).

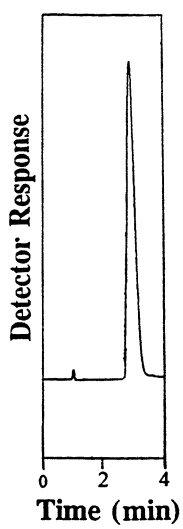


Fig. 4. A typical HPLC chromatogram of 20 μl injection of 8 μg $ml^{-1}$of lisinopril.

2.5. Standard solutions and calibration graphs

2.5.1. Spectrophotometric method

An accurately weighed quantity of 50 mg lisinopril dihydrate was dissolved in 10 ml 0.1 M sodium hydroxide (pH 13). Then, 8 ml of sodium hypochlorite solution was added. The solution was left for 5 min at room temperature. The volume was completed to 50 ml with sodium bisulphite solution. From this solution, different volumes (2–10 ml) were taken into a set of test tubes (20 ml capacity) and 4 ml of phenylhydrazine HCl solution were added. The contents were mixed and heated in a water bath at 85°C for 20 min. The contents were cooled and transferred quantitatively to 50-ml volumetric flasks and completed to volume using 0.5 M sodium hydroxide. The absorbance of the yellow color produced was measured at 362 nm against blank solution prepared similarly. The absorbance was plotted against the concentration. Linear relationship was obtained.

2.5.2. Spectrofluorimetric method

The standard solution of lisinopril dihydrate in the concentration of 1 μg ml^{-1} was prepared in distilled water. From this solution, different volumes (1–9 ml) were mixed with 3 ml *O*-phthalaldehyde buffered reagent in 50 ml volumetric flask. The mixture was left for 5 min, then the volume was completed to 50 ml with distilled water. The fluorescence intensity was measured at λ_{ex} 340 nm and λ_{em} 455 nm against blank solution prepared simultaneously. Calibration graph was constructed by plotting relative fluorescence intensity versus concentration. Linear relationship was obtained.

Table 2
Characteristic parameters for the regression equations of spectrophotometric (A), spectrofluorimetric (B) and HPLC (C) methods for determination of lisinopril

Parameters	A	B	C
Linearity	40–200 μg ml^{-1}	20–180 ng ml^{-1}	4–28 μg ml^{-1}
Regression equation (Y)[a]: slope (b)	5.80×10^{-3}	7.95×10^{-2}	2.38×10^{5}
Standard deviation of the slope (S_b)	4.41×10^{-5}	7.71×10^{-4}	2.86×10^{2}
Relative standard deviation of the slope (%)	0.76	0.97	0.12
Confidence limit of the slope[b]	5.71×10^{-3}–5.89×10^{-3}	7.79×10^{-2}–8.11×10^{-2}	2.37×10^{5}–2.39×10^{5}
Intercept (a)	-5.72×10^{-2}	7.86×10^{-2}	1.21×10^{3}
Standard deviation of the intercept (S_a)	5.90×10^{-3}	8.68×10^{-3}	5.32×10^{2}
Confidence limit of the intercept[b]	-4.45×10^{-2} –(-6.98×10^{-2})	6.00×10^{-2}–9.72×10^{-2}	7.15×10^{1}–2.35×10^{3}
Correlation coefficient (r)	0.9995	0.9996	0.9998
Standard error of estimation	8.30×10^{-2}	7.61×10^{-2}	6.31×10^{-2}

[a] $Y=a+bC$, where C is the concentration of lisinopril in μg ml^{-1} for A and C methods or ng ml^{-1} for B method; Y is the absorbance or relative fluorescence or peak area for A, B and C methods, respectively.
[b] 95% confidence limit.

Table 3
Determination of lisinopril in commercial tablets using spectrophotometric (A); spectrofluorimetric (B); HPLC (C) and official HPLC (D) methods

	Mean found ± S.D.[a]			
	A	B	C	D
Commercial tablets				
Zestril 5 mg	99.9 ± 0.85	100.5 ± 0.68	100.0 ± 0.55	100.1 ± 0.61
	$t=0.43$	0.98	0.27	$t=(2.31)$[b]
	$F=1.49$	1.4	1.23	$F=(6.39)$[b]
Zestril 10 mg	100.0 ± 1.46	99.9 ± 0.38	100.1 ± 0.62	100.2 ± 0.72
	$t=0.27$	0.82	0.24	$t=(2.31)$[b]
	$F=4.11$	3.59	1.35	$F=(6.39)$[b]
Zestril 20 mg	100.5 ± 0.89	99.5 ± 0.77	100.0 ± 0.76	99.9 ± 0.79
	$t=1.13$	0.82	0.20	$t=(2.31)$[b]
	$F=1.27$	1.05	1.08	$F=(6.39)$[b]
Recovery[c]				
Added to Zestril 5 mg	99.7 ± 1.34	99.8 ± 0.77	100.0 ± 0.69	
Added to Zestril 10 mg	99.8 ± 1.19	99.8 ± 0.92	100.1 ± 0.54	
Added to Zestril 20 mg	99.9 ± 1.20	100.0 ± 0.82	100.0 ± 0.67	

[a] Mean and S.D. for five determinations, percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of 50% of the nominal content ($n=5$).

2.5.3. HPLC method

The standard solutions of lisinopril dihydrate in the concentration range 4–28 μg ml^{-1} were prepared in mobile phase. Triplicate 20 μl injections were made for each concentration and chromatographed under the specified chromatographic conditions. Peak area values were plotted against concentration. Linear relationship was obtained.

2.6. Sample preparation

2.6.1. Spectrophotometric method

An accurately weighed quantity of the powder tablets equivalent to 50 mg lisinopril dihydrate was extracted with five 10 ml portions of methanol. The methanolic extracts were filtered and evaporated to dryness in a water bath at 40°C. The residue was treated as under calibration graphs.

2.6.2. Spectrofluorimetric and HPLC methods

An accurately weighed quantity of the powder tablets equivalent to 20 mg lisinopril dihydrate was extracted with 100 ml distilled water and filtered. Further dilutions of the filtrate were made with distilled water (for spectrofluorimetric method) or mobile phase (for HPLC method) to suit each method. The general procedures for both methods described under calibration were followed.

2.7. Percent recovery study

This study was performed by addition of known amount of lisinopril dihydrate to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and results obtained were compared with expected results (Table 3).

3. Results and discussion

3.1. Spectrophotometric method

The UV absorption spectrum of lisinopril in 0.1 M hydrochloric acid has no characteristic absorption band (Fig. 1), in addition the drug is weakly absorbing in the UV region (A 1%/1 Cm values are 3.2, 3.9, 4.5, 3.0 and 2.8 at 246, 253, 258, 264 and 267 nm, respectively) [16]. Hence, conventional spectrophotometric method cannot be applied for its determination due to interference from formulation matrix. The proposed spectrophotometric method depends on derivatization of lisinopril after conversion of its secondary amino group by sodium hypochlorite into 1-(6-amino-2-oxo-hexanoyl)-2-carboxypyrolidine and 2-amino-4-phenyl butyric acid. The excess sodium hypochlorite was removed by the addition of sodium bisulphite. The derivatization was carried out through the condensation of the resulted carbonyl compound with phenylhydrazine by heating at 85°C for 20 min (Scheme 1). The absorbance of yellow colored product [1-(6-amino-2-phenylhydrazono-hexanoyl)-2-carboxypyrolidine] was measured at 362 nm in 0.5 M sodium hydroxide (Fig. 2).

The conversion reaction conditions were studied as a function of the pH, reaction time, sodium hypochlorite and sodium bisulphite concentrations. Also, the condensation reaction conditions were studied as a function of the phenylhydrazine concentration, reaction temperature and reaction time. The described procedure gives maximum stability and sensitivity.

It has been reported that lisinopril decomposition proceeds rapidly in acidic media with the major decomposition product being the diketopiperazine. In neutral and basic media, the decomposition rate is minimal [16]. The use of A_{max} method for the determination of the intact drug in presence of its acid-induced degradation product will give unacceptable results due to the spectral overlapping of the degraded product [4]. The proposed spectrophotometric method was extended to develop a stability indicating method for the determination of intact lisinopril in presence of its acid-induced degradation product. Where the principal degradation product, diketopiperazine, a product of intramolecular dehydration between secondary amino group and the carboxylic group at the proline, does not give the condensation reaction with phenylhydrazine (Fig. 2).

The accuracy of the proposed spectrophotometric method was checked by analyzing eight laboratory prepared mixtures of lisinopril and its acid-induced degradation product, diketopiperazine, at various concentrations ranged from 100 to 200 μg ml^{-1} for lisinopril and 2–40 μg ml^{-1} for diketopiperazine. The mean percentage recovery ± S.D. for lisinopril was found to be 99.94 ± 1.11 (Table 1). This indicates the high repeatability and accuracy of the method. The spectrophotometric method able to determined

lisinopril in presence of diketopiperazine in ratio up to 1:0.2, respectively.

3.2. Spectrofluorimetric method

Lisinopril, as amino acid derivative containing primary amine, it reacts with *O*-phthalaldehyde in borate buffer pH 9.5 in presence of 2-mercaptoethanol as stabilizing agent to form a product exhibits strong fluorescence at 455 nm upon excitation at a maximum of 340 nm (Scheme 2) (Fig. 3). This permits the development of a very sensitive method of assay for lisinopril in its tablets. The different parameters affecting the fluorescence development such as pH of the buffer, reaction time, *O*-phthalaldehyde and 2-mercaptoethanol concentrations were studied so as to give the best sensitivity and stability. This study led to the described procedure. Due to the high sensitivity of the spectroflourimetric method, it can be used for determination of very low concentration of lisinopril. Also, it is used for determination of the drug in pharmaceutical tablets.

3.3. HPLC method

Peak splitting in reversed phase liquid chromatography of lisinopril was observed. High column temperature and low pH values of the mobile phases were found to improve the peak shape and resolution [5]. Recently, several amines have been analyzed on silica-based systems with aqueous mobile phases [18]. This approach has been adopted for the analysis of lisinopril. The developed HPLC method based on using Hypersil Silica column, with a mobile phase consisting of methanol–water–triethylamine in a ratio 50:50:0.1 v/v and the pH was adjusted to 2.6 with 0.1 N perchloric acid with flow rate of 1 ml min^{-1} at ambient temperature. The effects of mobile phase composition and pH on chromatographic separation of lisinopril were studied and optimized. The addition of triethylamine to the mobile phase was essential to improve the sharpness of the lisinopril peak. Quantitation was achieved with UV detection at 210 nm based on peak area. Under the described chromatographic conditions, sharp peak was obtained for lisinopril without any splitting or broadening (Fig. 4). The average retention time $\pm$ S.D. for lisinopril was found to be 2.9 ± 0.007 min, for ten replicates.

Under the described experimental conditions of the above mentioned three methods, plots of absorbance, relative fluorescence and peak area values versus concentrations within the range stated in the Table 2 show linear relationships. The regression analysis of these plots using the method of least squares was made (Table 2). The linearity of the calibration graphs was validated by the high values of correlation coefficients of the regression equations.

3.4. Method validation

Spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels: 50, 75, 100, 125 and 150% of label claim for the drug. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for the drug, determined by the proposed methods. Spiked placebo assays were used to determine accuracy and precision of the proposed methods for determination of the drug. The recoveries ranging from 99.7 to 100.5% of the amount of active ingredient spiked into the placebo. The bias showed only minor variation in recovery at each level with 0.5% the maximum variation observed. The proposed methods were tested for repeatability, reproducibility, selectivity, specificity, robustness and ruggedness. Satisfactory results were obtained. The proposed methods complied with USP [3] validation guidelines.

The non-instrumental methods for determination of the detection limit and the quantitation limit were applied [3], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantitation is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with accept-

able accuracy and precision. The detection limits of the proposed methods were found to be 5, 0.003 and 0.7 μg ml^{-1} for lisinopril detected by spectrophotometric, spectroflourimetric and HPLC methods, respectively. While the quantitation limits of the proposed methods were found to be 20, 0.01 and 1 μg ml^{-1} for lisinopril determined by spectrophotometric, spectroflourimetric and HPLC methods, respectively.

The stability of lisinopril dihydrate during the analytical procedures was studied and found to be stable. The analyte was stable for at least 24 h in solution.

3.5. Tablet analyses

The three proposed methods were applied to the determination of lisinopril in commercial tablets. Five replicates determinations were made. Satisfactory results were obtained and were in a good agreement with the label claims (Table 3). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding lisinopril to the earlier analyzed tablets. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analyses of the commercial tablets and the recovery study (standard addition method) of the drug (Table 3) suggested that there is no interference from any excipients, which are present in tablets. The results of determination of lisinopril in tablets obtained from the spectrophotometric, spectrofluorimetric and HPLC methods were compared with those of the official HPLC method [3] using octylsilane column at 50°C and phosphate solution–acetonitrile (96:4 v/v) as mobile phase. Statistical comparison of the results was performed with regard to accuracy and precision using Student's *t*-test and *F*-ratio at 95% confidence level (Table 3). There is no significant difference between the proposed methods and official HPLC method with regard to accuracy and precision. Also, the results of determination of lisinopril in tablets obtained from the three proposed methods were compared with those of the HPTLC method carried out on Merck HPTLC aluminum plates of silica gel 60 F_{254} using chloroform–ethylacetate–acetic acid (10:3:2 v/v) as mobile phase, followed by densitometric measurement of the spot at 210 nm; and third derivative spectrophotometric method measured at 217.4 nm in 0.1 M hydrochloric acid. Similar results were obtained without any significance difference with regard to accuracy and precision.

4. Conclusion

The proposed spectrophotometric, spectrofluorimetric and HPLC methods provide simple, accurate and reproducible quantitative analyses for the assay of lisinopril in tablets. The three methods overcome the problem of low absorptivity of the drug in UV region. The spectrophotometric method is a stability indicating method. While the spectrofluorimetric method has the greatest sensitivity. The HPLC method is more specific than the other two methods. The possibility of the use of spectroflourimetric and HPLC method as stability indicating method was not studied. The proposed HPLC method can be applied at ambient temperature while other published HPLC method were applied at elevated column temperature. The spectroflourimetric method is more sensitive than other published methods for determination of lisinopril.

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