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Note

NOTE

Determination of lisinopril in pharmaceuticals by a kinetic spectrophotometric method

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Abstract: A kinetic spectrophotometric method for determination of lisinopril in pharmaceuticals has been developed. The method is based on the activator action of lisinopril on Cu(II) ions catalysing the oxidation of Nile Blue A with hydrogen peroxide in borate buffer (pH 9.3). A decrease of the absorbance was recorded at 635 nm after 5 min at 25 °C. Linearity was established by application of the tangent method within the concentration range of lisinopril from 0.8–6.4 μg mL^{-1}, the detection and quantification limits being 0.158 and 0.480 μg mL^{-1}, respectively. The method was successfully applied to three brands of tablets containing lisinopril alone or in combination with hydrochlorothiazide.

Keywords: lisinopril; Nile Blue A; spectrophotometry; kinetic determination; pharmaceuticals.

INTRODUCTION

Lisinopril, (*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline, belongs to the group of angiotensin converting enzyme (ACE) inhibitors, widely applied in the treatment of high blood pressure.[1] Due to its wide application in medicine, there is an increasing interest for the development of sensitive and highly selective methods for lisinopril determination in pharmaceuticals and biological materials. Several spectrophotometric[2–16] and spectrofluorimetric[3,17] procedures have been described for lisinopril determination. Thus, zero order[5] and derivative spectrophotometry[2,7–9] have been proposed for the direct determination of lisinopril. Considering the very low absorbance of lisinopril in the UV region, some authors have applied derivatisation in order to increase the sensitivity of the determination.[3,4,6,10–16] The methods based on derivatisation are time consuming, usually require heating and cooling of the reaction mixture and the use of organic

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solvents. Furthermore, finding out an appropriate reagent that would quantitatively react with the analysed substance represents an additional problem of derivatisation. Of all the spectrophotometric methods hitherto proposed for lisinopril determination, only Rahman *et al.*[12] recently reported a kinetic procedure.

The present study was aimed at the development of a rapid and sensitive kinetic spectrophotometric method for lisinopril determination in pharmaceuticals. The procedure is based on the activator effect of lisinopril on the oxidation reaction of Nile Blue A with hydrogen peroxide, catalysed by Cu(II) ions. The method is more sensitive than the already reported spectrophotometric approaches, which are listed in the literature,[15] and lisinopril is determined avoiding derivatisation and the use of organic solvents.

EXPERIMENTAL

Apparatus

A GBC Cintra 20 spectrophotometer (GBC Scientific Equipment Pty Ltd., Dandenog, Australia) with 1.0 cm quartz cuvettes and software for kinetic measurements was used. The temperature of the samples was maintained at 25±0.1 °C using a Huber Polistat CC2 thermostat.

Materials and reagents

Lisinopril dihydrate and hydrochlorothiazide were kindly provided by the Medicines and Medical Devices Agency of Serbia (Belgrade, Serbia) and Zdravlje Actavis Company (Leskovac, Serbia), respectively. Water content in lisinopril dihydrate standard was determined by titrimetry at time of use for analysis. Pharmaceutical lisinopril preparations, such as Loril® (Srbolek, Serbia), Skopryl® (Alkaloid, Macedonia) and Lizopril® H (Bosnalijek, Bosnia and Herzegovina) were purchased from a local market. Claimed lisinopril content per tablet in the examined samples was 5 mg lisinopril dihydrate (Loril®), *i.e.*, 10 mg lisinopril (Skopryl®) and 10 mg lisinopril dihydrate plus 12.5 mg hydrochlorothiazide (Lizopril® H). Nile Blue A (Sigma–Aldrich), hydrogen peroxide 30 % (Merck), disodium tetraborate decahydrate (Baker) and anhydrous copper(II) sulphate (Merck) were of analytical reagent grade. Other reagents used throughout the present study were of analytical grade purity. All solutions were prepared in double distilled water. The concentrations of the stock solutions were: Nile Blue A, 1.00×10^{-4} mol L^{-1}, and Cu(II) sulphate, 1.00×10^{-4} mol L^{-1}. Borate buffer (0.1 mol L^{-1}), pH 9.3, was prepared by dissolving sodium tetraborate in water. Hydrogen peroxide solution (3.92×10^{-1} mol L^{-1}) was obtained by diluting 30 % H_2O_2 with water.

Procedure

The reaction was performed in a reaction-mixture vessel with three compartments for rapid mixing of the stock solutions, designed by L.I. Budarin.[18] The measured amounts of Nile Blue A and Cu(II) solutions were placed in the first compartment of the vessel, and borate buffer, lisinopril solution and water (up to a total volume of 25 mL) in the second compartment. After thermostating at 25 °C, hydrogen peroxide solution was added into the third compartment and the reaction initiated by mixing the reactants (zero time). After 30 s, the absorbance was recorded at 635 nm for 5 min at 30 s intervals. To study the effects of hydrochlorothiazide on the determination of lisinopril, a solution of hydrochlorothiazide was stored in lisinopril-containing compartment.

Calibration curve. A calibration curve was obtained by the described procedure for the following amounts of the reagents: 0.8 mL hydrogen peroxide (3.92×10^{-1} mol L^{-1}), 5 mL 1.00×10^{-4} mol L^{-1} Nile Blue A solution, 4 mL 1.00×10^{-4} mol L^{-1} Cu(II) solution, 5 mL borate buffer, pH 9.3 (0.1 mol L^{-1}), aliquots of 0.5–4.0 mL of the solution containing 40 μg mL^{-1} lisinopril and water up to a total volume of 25 mL.

Analysis of pharmaceuticals. Twenty lisinopril-containing tablets were precisely weighed and pulverised. The amount of the obtained powder corresponding to the mass of a single tablet was transferred to a 100 mL volumetric flask and water added up to the volume. The mixture was treated for 10 min in an ultrasonic bath and filtered. For further analyses, 0.8 mL (Skopryl® and Lizopril® H tablets) and 1.6 mL (Loril® tablets) aliquots of the resulting filtrates were used.

RESULTS AND DISCUSSION

The presence of Cu(II) ions decreased the absorbance in the system Nile Blue A – hydrogen peroxide, thus demonstrating that copper ions acted as a catalyst of this reaction. Addition of lisinopril to this system led to a further decrease of the absorbance, indicating an increased reaction rate and demonstrating the activator action of lisinopril. In order to optimise experimental conditions for lisinopril determination, the kinetics of the catalytic reaction of the system Nile blue A – hydrogen peroxide – Cu(II), in the presence and in the absence of lisinopril, was examined. The reaction rate was recorded spectrophotometrically at 635 nm, applying the tangent method.[18] The concentration ranges of the reagents and selected optimal conditions for lisinopril determination are listed in Table I.

TABLE I. Concentration range and optimal concentrations of the reagents for the determination of lisinopril by the proposed kinetic spectrophotometric method

Reagent	Range	Optimal values
Cu(II), mol L^{-1}	0.40×10^{-5}–2.00×10^{-5}	1.60×10^{-5}
H_2O_2, mol L^{-1}	0.625×10^{-2}–1.57×10^{-2}	1.25×10^{-2}
Borate buffer, pH	8.6–9.3	9.3
Borate buffer (pH 9.3), mol L^{-1}	1.00×10^{-2}–3.00×10^{-2}	2.00×10^{-2}

A linear relationship was established within lisinopril concentration range 0.80–6.40 μg mL^{-1} (0.197×10^{-5}–1.58×10^{-5} mol L^{-1}), and the parameters obtained by regression analysis are given in Table II. The precision of the method was estimated by performing six determinations of 1.60, 3.20 and 5.60 μg mL^{-1} lisinopril. The results obtained are listed in Table III. The accuracy of the method was established by performing recovery experiments at three levels (by adding 80, 100 and 120 % lisinopril of the claimed tablet content) using the standard addition method. The analysed samples, Skopryl® and Loril® tablets, were spiked with additional 8, 10 and 12 mg, *i.e.*, 4, 5 and 6 mg lisinopril, respectively, and the content of lisinopril determined by the proposed method. The recoveries ranged from 99.16 to 101.4 % (Table IV).

The proposed method was applied to the determination of the lisinopril content in three different commercial tablets (loril, skopryl and lizopril H).

TABLE II. Statistical data of lisinopril determination by the proposed kinetic spectrophotometric method (n = 6)

Parameter	Value
Concentration range, μg mL^{-1}	0.80–6.40
Calibration equation	$2.097\times10^{-4} + 1.578\times10^{-5}c$
Correlation coefficient (r)	0.9997
Standard deviation of slope	1.81×10^{-7}
Standard deviation of intercept	7.57×10^{-7}
Limit of detection (LOD / μg mL^{-1})	0.158
Limit of quantification (LOQ / μg mL^{-1})	0.480

TABLE III. Precision test of the proposed kinetic spectrophotometric method for lisinopril determination (six independent determinations)

Analysed lisinopril amount, μg mL^{-1}	SD	RSD / %	Standard analytical error
1.60	0.049	3.15	0.020
3.20	0.040	1.22	0.016
5.60	0.028	0.48	0.011

TABLE IV. Accuracy of the proposed kinetic spectrophotometric method for the determination of lisinopril (n = 6)

Pharmaceutical formulation analyzed	Amount of lisinopril, mg					
	In analysed tablets	Standard added	Total	SD	RSD / %	Recovery, %
Skopryl	9.850	8.00	17.94	0.095	1.17	101.1
	9.850	10.00	19.78	0.127	1.28	99.30
	9.850	12.00	21.75	0.027	0.23	99.16
Loril	4.544	4.00	8.601	0.088	2.17	101.4
	4.544	5.00	9.522	0.092	1.85	99.56
	4.544	6.00	10.55	0.115	1.91	100.1

The selectivity of the proposed method was investigated by the determination of 4 μg mL^{-1} lisinopril solution in the presence of various water soluble compounds commonly found in lisinopril tablets within a relative error of ±5 %. The co-existing soluble ingredients did not interfere with the determination (mass ratio to lisinopril): Ca^{2+} (1000); HPO_4^{2-} (100) and mannitol (10 000). Since lizopril H tablets in addition to lisinopril (10 mg in the form of dihydrate) also contain hydrochlorothiazide (12.5 mg), the effects of the latter component on the determination of lisinopril by the proposed kinetic method were examined. The results clearly showed that hydrocholorothiazide did not interfere up to the mass ratio lisinopril:hydrochlorothiazide of 1:1.4. Since commercially available pharmaceutical preparations contain at the most a 1.25 higher content of hydrochlorothiazide than lisinopril, the proposed kinetic procedure described in the present

work could be successfully applied for the determination of lisinopril in these preparations. The obtained results of lisinopril determination in tablets are summarized in Table V.

TABLE V. Determination of lisinopril in commercial tablets by the proposed kinetic spectrophotometric method (n = 6)

Commercial tablets	Claimed content *per* tablet, mg	Found mg	Found content calculated as lisinopril dihydrate, mg	In relation to claimed content, %
Loril	5[a]	4.544[b]	4.947	98.94
Skopryl	10[b]	9.850[b]	–	98.50
Lizopril H	10[a]	9.286[b]	10.11	101.1

[a]Lisinopril dehydrate; [b]lisinopril

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И З В О Д

ОДРЕЂИВАЊЕ ЛИЗИНОПРИЛА У ФАРМАЦЕУТСКИМ ПРЕПАРАТИМА ПРИМЕНОМ КИНЕТИЧКЕ СПЕКТРОФОТОМЕТРИЈСКЕ МЕТОДЕ

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Развијена је кинетичка спектрофотометријска метода за одређивање лизиноприла у фармацеутским препаратима. Метода се заснива на активаторском дејству лизиноприла на реакцију оксидације боје нил плавог-А водоник-пероксидом у боратном пуферу (pH 9,3), а која је катализована Cu(II) јонима. Смањење апсорбанције мерено је на 635 nm у временском периоду од 5 мин на температури 25 °C. Линеарност је утврђена применом методе тангенса у опсегу концентрација лизиноприла 0,8–6,4 μg mL^{-1}, са лимитом детекције 0,158 μg mL^{-1} и лимитом одређивања 0,480 μg mL^{-1}. Метода је примењена за анализу три комерцијална препарата која су садржала лизиноприл и лизиноприл у комбинацији са хидрохлортиазидом.

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