A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF LISINOPRIL IN TABLET FORMULATION

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ABSTRACT

A simple and sensitive spectrophotometric method is presented for the assay of lisinopril in tablets. The method involves the formation of hydroxamate by the reaction of carboxyl group with hydroxylamine hydrochloride (HAHC) and dicyclohexylcarbodiimide (DCC) and the formation of the reddish brown coloured iron(III) hydroxamate complex with iron (III) perchlorate in ethanolic solution of perchloric acid. The formation of hydroxamate was completed in 10 min. at 40 °C with 0.5 ml of 0.3 mol L^{-1} ethanolic hydroxylamine hydrochloride and 0.5 ml of 0.3 mol L^{-1} ethanolic DCC solutions for lisinopril. 1.0 ml of 0.02 mol L^{-1} iron (III) perchlorate in 0.2 mol L^{-1} ethanolic perchloric acid solution were required for complex formation. The complex has shown maximum absorbance at 506 nm. The quantification limit is 2.759 x 10^{-5} mol L^{-1} (11.174 μg mL^{-1}) at 506 nm. The limit of detection is 1.403 x 10^{-6} mol L^{-1} (0.568 μg mL^{-1}) at 506 nm. The method was applied to commercially available tablets and the results were statistically compared with those obtained by an UV spectrophotometric method using t- and F- tests at 95 % confidence level.

Keywords: Lisinopril determination, dicyclohexylcarbodiimide, hydroxylamine hydrochloride, iron(III) hydroxamate complex, spectrophotometry

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INTRODUCTION

Lisinopril, [(S) – 1 – {N- (1 – carboxy – 3 – phenylpropyl) – L – lysyl } – L – proline dihydrate], a lysine analogue of enalaprilat, is a long acting and non sulfhydryl angiotensin converting enzyme inhibitor and used for the treatment of hypertension and congestive heart failure.

Some methods have been reported for the quantitation of lisinopril. A gas liquid chromatographic estimation in pharmaceutical dosage forms /1/, some determinations in human plasma by gas chromatography – mass spectrometry /2,3/, a polarographic study in pharmaceutical formulation /4/, an assay of lisinopril binding to angiotensin I – converting enzyme by colorimetric analysis /5/, some spectrophotometric determinations in pharmaceutical dosage forms based on the reactions with : 2,4,6- trinitrobenzoic acid /6/, 1,2 – naphthoquinone – 4 – sulphonic acid sodium salt (NQS) /7/, chloranil in aq. solution at pH 9.5 to give yellow coloured product and diclone to give an intense purple coloured product /8/, sodium hypochloride and phenyl hydrazine to give a condensation product /9/, 1-fluoro-2,4-dinitrobenzene /10/, some simultaneous spectrophotometric determinations in binary dosage formulations based on development of equations for the area calculation of spectrum at 2 wavelength regions /11,12/, an UV absorption method /13/, some derivative spectrophotometric studies in combine dosage forms /8,14-18/, spectrofluorometric assays in pharmaceutical tablets and biological fluids /8,9,19,20/, a time resolved fluoroimmuno assay in human serum /21/, a radioimmuno assay (RIA) /22/, densitometric determination in binary mixtures /14/, some liquid chromatographic analysis in pharmaceutical formulations and human urine /9,17;23–26/, determinations by capillary electrophoresis in pharmaceutical formulations /27 – 29/ have been reported.

Ferric hydroxamate reaction allows spectrophotometric determination of carboxylic acids after conversion into corresponding hydroxamic acids. Dicyclohexylcarbodiimide (DCC) have been used for the formation of hydroxamic acids from free carboxylic acids and hydroxylamine /30,31/.

Recently, we used ferric hydroxamate reaction in our previous study for the spectrophotometric determination of cilazapril /32/ which has a similar structure to that of lisinopril, in pharmaceutical preparations. Satisfactory analysis results have been obtained.

EXPERIMENTAL

Materials

Pharmaceutical grade lisinopril was donated from İlsan – İltaş A.Ş. Istanbul, Turkey. Iron(III) perchlorate was obtained from Fluka Chemie AG, Switzerland and other chemicals from Merck, Darmstadt, Germany were all of analytical reagent grade. Absorbance values were measured with a UV Visible double beam spectrophotometer (Schimadzu UV 150 02) and 1 cm glass cells. 0.3 mol L^{-1} hydroxylamine hydrochloride and 0.3 mol L^{-1} DCC solutions in ethanol were used. 0.02 mol L^{-1} iron (III) perchlorate solution was prepared in 0.2 mol L^{-1} ethanolic perchloric acid solution. Standard lisinopril solution (3.0 mg mL^{-1}, in methanol) was used for the establishment of the standard curve. Sample solutions were obtained from tablet powders equivalent to (80 – 90) mg of lisinopril, by extraction with methanol in 50 ml flask, dilution to 50 ml with the same solvent and filtration with quantitative filter paper.

Assay Procedure

(0.2 – 1.0) ml of standard lisinopril solution for establishment of the calibration curve or 1.0 ml of the sample solution was transferred into a set of test tubes. After dilution to 1.0 ml with methanol, 0.5 ml of DCC and 0.5 ml of HAHC solutions were added and mixed. The mixtures were heated at 40 °C for 10 min. After cooling to ambient temperature, the contents were transferred quantitatively to 10 ml volumetric flasks. 1.0 ml of iron (III) perchlorate solution was added and diluted to volume with ethanol. The absorbance of the solutions was measured at 506 nm against blank. The amount of lisinopril in eachtablet was calculated by means of the regression equation of the standard curve obtained by standard solutions.

RESULTS AND DISCUSSION

A new and selective spectrophotometric method has been developed for the analysis of lisinopril by means of a derivatization reaction based on the formation of ferric hydroxamate complex of lisinopril.

Ferric hydroxamate reactions have been formed by means of the reaction of hydroxamic acids and Fe^{3+} ion. Hydroxamic acids are obtained with the

reaction of carboxyl groups and hydroxylamine. As carboxylic acids can not give a direct reaction with hydroxylamine for the step of formation of hydroxamic acids they must be converted to their esters beforehand for the formation of hydroxamate reaction. Dicyclohexylcarbodiimide has been used for esterification of carboxyl group in the structure of lisinopril.

Optimum conditions of the hydroxamate reactions were investigated in respect to the amounts of DCC, hydroxylamine hydrochloride and iron (III) perchlorate, temperature, time and acidity.

It has been considered that the formation of hydroxamic acid with the reaction of drug substance, DCC and hydroxylamine has been carried out as below (Figure 1).

As seen in Figures 2-4, 0.5 ml of 0.3 mol L^{-1} dicyclohexylcarbodiimide, 0.5 ml of 0.3 mol L^{-1} hydroxylamine hydrochloride, 1.0 ml of 0.02 mol L^{-1} iron(III) perchlorate were found to be sufficient for the formation of lisinopril hydroxamate complex.The optimum temperature and time were determined as 10 min at 40 °C for the formation of lisinopril hydroxamate complex according to Figure 5. Effect of the acidity was searched on the formation of complex by using 0.1, 0.2 and 0.3 mol L^{-1} perchloric acid solutions.

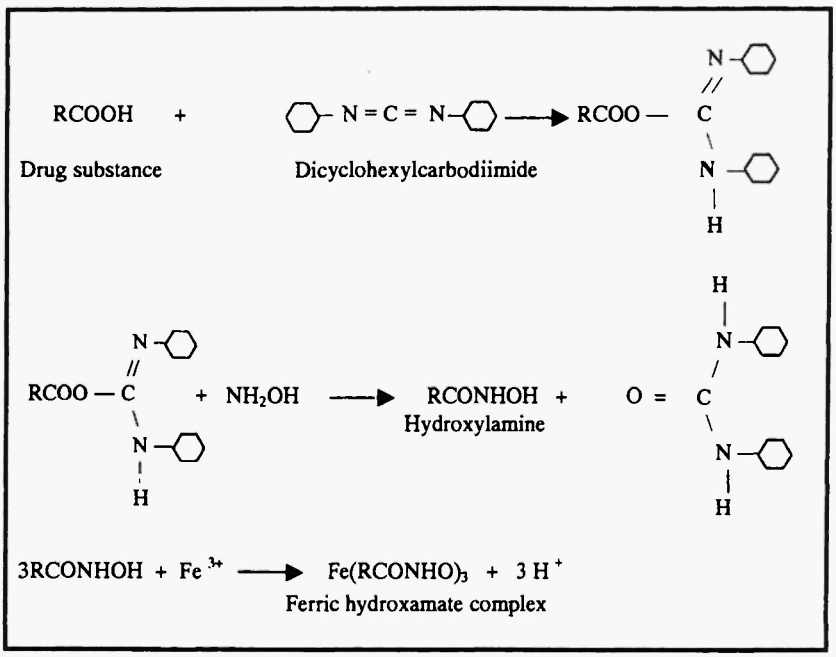

Fig. 1: The equations of reaction on the formation of lisinopril hydroxamate

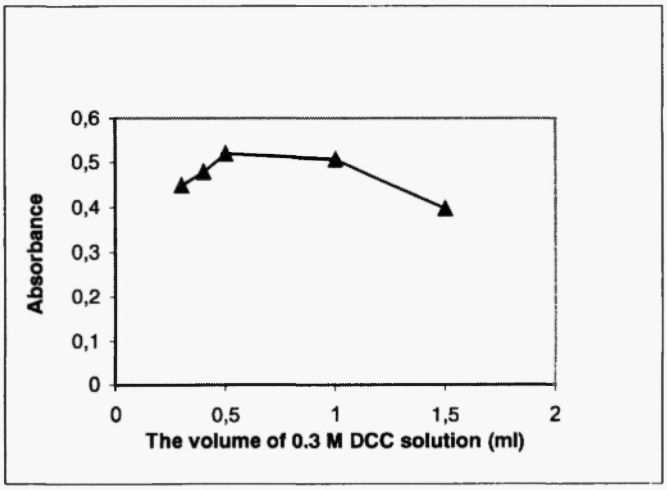

Fig. 2: Effect of dicyclohexylcarbodiimide amount on the formation of the hydroxamate complex

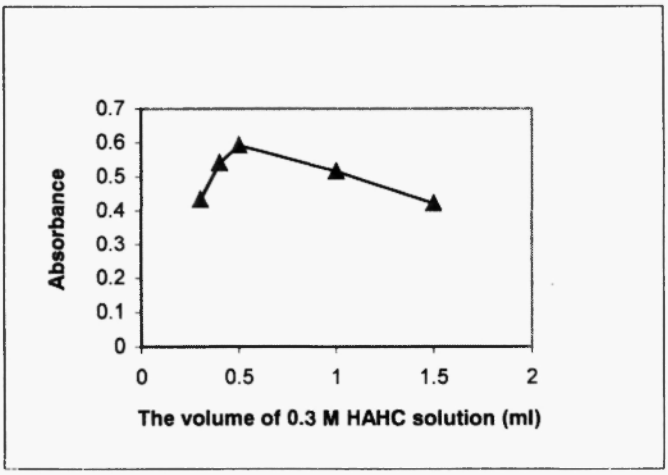

Fig. 3: Effect of hydroxylamine hydrochloride amount on the formation of the hydroxamate complex

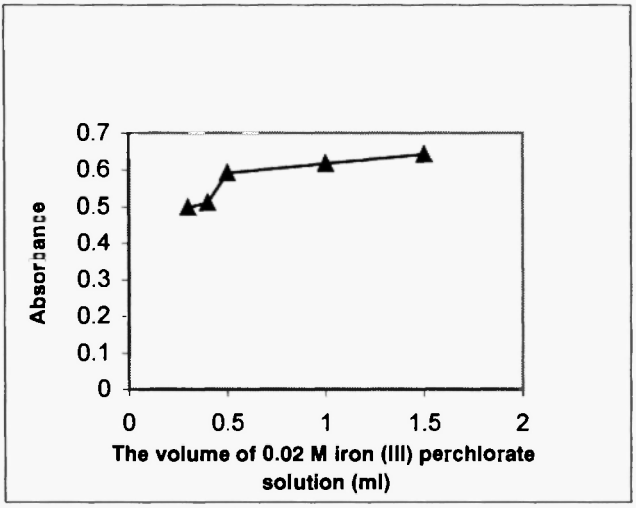

Fig. 4: Effect of 0.02 mol L^{-1} iron (III) perchlorate amount on the formation of the hydroxamate complex

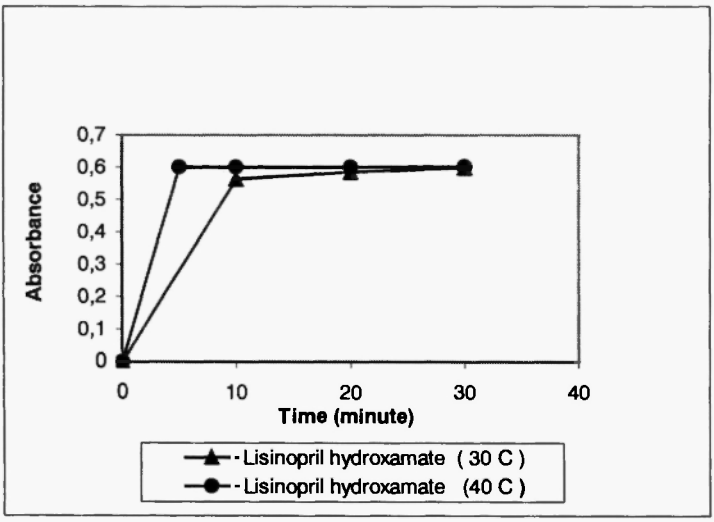

Fig. 5: Effect of temperature and time on the formation of lisinopril hydroxamate complex

Satisfactory results were obtained with 0.02 mol L^{-1} iron (III) perchlorate solution in 0.2 mol L^{-1} perchloric acid solution.

Under the experimental conditions described above, a linear relationship was obtained between absorbance (A) and lisinopril concentration (C) in the range of 60-300 μg mL^{-1}.

The regression equation of the calibration curve was calculated as:

$$A = 0.00264\ C + 0.0105 \quad (r = 0.9996\ ;\ C = \mu g\ mL^{-1})$$

The quantification and detection limits of the method were estimated from the mean equations of the calibration curves based on the standard deviation of the intercepts. The quantification limit (based on 10 times the SD) was found equal to 11.174 μg mL^{-1} at 506 nm.The detection limit (based on 3 times the SD) was found equal to 0.568 μg mL^{-1} at 506 nm.

The limit of quantification (LOQ) and the limit of detection (LOD) values of the UV spectrophotometric method were calculated at 215 nm respectively 18.236 μg mL^{-1} and 5.468 μg mL^{-1}.

The spectrophotometric method was applied to the commercially available tablets (Acerilin). Satisfactory results were obtained for tablet formulation. The assay results are given in Table 1. The results were compared with those obtained by UV spectrophotometric method in terms of t- and F- tests.

The UV spectrophotometric method was applied to compare the results as it is simple and rapid. The method was applied as single standard method. It was based on the measurement of absorbances of standard lisinopril solution (10 μg mL^{-1}; in methanol) and sample solution obtained from tablet powders at 215 nm. As seen from Table 1, at 95 % confidence level, there was no significant difference between the mean values and standard deviations of both methods.

Commonly used tablet ingredients do not have interfering effects on the method. The proposed method has been applied as well as the spectrophotometric determination of cilazapril /33/ and lisinopril in pharmaceutical formulations and satisfactory results have been obtained for both drug substances. On the other hand the tablet ingredients do not have interference effect on the UV spectrophotometric method as they do not have spectacular absorbance at 215 nm.

Table I

Assay results of lisinopril tablet [Acerilin(İlsan-Iltaş A.Ş.)] ; 20 mg/tablet]

Statistical Value	Described Method (mg)	UV Spectrophotometric Method* (mg)
Found Mean Amount (mg)	20.7	20.6
Confidence Limits of the Found Mean lisinopril (mg)	20.619 – 20.781	20.566 – 20.633
Mean Recovery (%)	100.72	101.83
Standard Deviation	0.79	0.33
Relative Standard Deviation (%)	3.82	1.60
The Number of Determinations	5	5
t test of significance	t = 0.23 (p= 0.05 ; t = 2.31)	
F test of significance	F = 5.83 (p= 0.05 ; F = 6.39)	

*λ_{max} = 215 nm in methanol

CONCLUSION

The proposed method is a selective method to determine drug substances containing free carboxyl group like lisinopril. Although its sensitivity is not very high, it is more sensitive than the UV spectrophotometric method. The method is simple and rapid according to other spectrophotometric methods /8-10/ in literature. The procedure takes only 10 minutes and can be applied in the presence of substances containing an amine group like hydrochlorothiazide. There are commercial preparations containing binary mixtures of lisinopril and hydrochlorothiazide such as zestoretic, sinoretik

and rilace plus. The method is selective to determine lisinopril near hydrochlorothiazide in this preparations. The determination of cilazapril in pharmaceutical formulations by the proposed method has given accurate results /32/.

Y. Kasai *et al.* /31/ has shown that iron (III) hydroxamate complex is stable in perchloric acid and perchlorate ion has the ability of coordination, in the presence of complexing agents which can react with iron. It is expected that the good results will be obtained by this method by means of free perchloric acidic media.

Spectrophotometric determinations of lisinopril in literature have commonly been based on the reaction of the amine group in its structure /7,8,10/. The proposed method has been introduced as a new approach in respect to spectrophotometric determination of lisinopril based on derivatisation of its carboxyl group.

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