HIGH PRESSURE LIQUID CHROMATOGRAPHIC INVESTIGATION OF THE pH AND TEMPERATURE EFFECT ON THE SOLUTION STABILITY OF LISINOPRIL

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ABSTRACT

Lisinopril, a new drug substance, is an angiotensin-converting enzyme (ACE) inhibitor. In this study, we investigate the high pressure liquid chromatographic pH effect on the solution stability of lisinopril. Lisinopril solutions of 75 µg/ml were prepared in various buffer solutions in the range of pH = 2-8. These solutions were heated in an oven at 80°C for various hours. After these periods the solutions were removed and cooled immediately. Then, 10µl of the solution was injected into a high pressure liquid chromatographic system containing a Bondapak C18 reversed phase column and a variable wavelength UV – visible absorbance detector. The separation was performed with pH = 5 phosphate buffer – acetonitril – triethylamine (90 : 10 : 0.1) mixture as isocratic mobile phase. The detector was operated at 209 nm. The investigation of the chromatograms and lisinopril peak areas indicated that pH has a significant effect on the stability of lisinopril in aqueous solution and decomposition rate of lisinopril is increased by the decreasing pH. Pseudo first order rate constants were calculated using the time – log (peak area) plots.

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INTRODUCTION

Lisinopril, (S)-1-[N²-(1-Carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate, is an angiotensin converting enzyme (ACE) 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 dichlorone and acetylacetone with formaldehyde /4/ and 2-mercaptoethanol in borate buffer pH 9,5 with o-phthalaldehyde /5/. First, second derivative spectrophotometric /4,6/ and spectrofluorometric methods /4-5,7/. The chromatographic techniques of analyses, HPLC using different reversed phase columns /5-6,8-11/ and gas liquid chromatography /12/ have been employed. Capillary electrophoresis was applied to determination of lisinopril in pharmaceutical tablets /13/. The literature presents few methods for determination of lisinopril in biological fluids. These include fluoroimmunoassay /14/, radioimmunoassay /15/, fluoroenzymatic assay /16/ and polarographic method /17/. Among these techniques, HPLC is the most convenient procedure for the stability studies. The others are not specific for stability analysis of lisinopril in drug products.

The aim of this study is the high pressure liquid chromatographic investigation of the pH and temperature effect on the solution stability of lisinopril.

EXPERIMENTAL

Chemicals and Reagents

All chemicals were analytical reagent grade. Mobile phase solvents acetonitrile and methanol were HPLC grade; and obtained from J.T. Baker. Other analytical grade chemicals were purchased from E. Merck. MilliQ water was used. Lisinopril (Ilsan/Istanbul) was used as received.

Standard lisinopril solution ($1000\mu\text{g.mL}^{-1}$) was prepared by dissolving 0.01 g of lisinopril in water and diluted to 10 mL with the same solvent.

Buffer solutions of pH= 2.0 – 8.0 were prepared by mixing appropriate volumes of 0.02 M H_3PO_4 , NaH_2PO_4 , Na_2HPO_4 and Na_3PO_4 solutions. Ionic strength of these buffers were adjusted to 0.03 with the help of KCl solution.

Instrumentation

The HPLC system consisting of the components: a C_{18} 10 μm Bondapak column (300x3.9 mm i.d), a Model 510 delivery system and a model 481 variable wavelength spectrophotometer was from Waters (U.S.A.). Absorption spectra were recorded with Philips 8740 spectrophotometer and 1 mm quartz cells.

HPLC Conditions

The mobile phase was composed of 0.02 M pH=5 phosphate buffer / acetonitrile / triethylamine (90:10:0.1), degassed using ultrasonic bath and filtered through 0.45 μm filter. The eluent flow rate was 1 mL/min (1100 PSI). Sample injection volume was 10 μL .

Procedure

A number of solutions containing 75 $\mu\text{g.mL}^{-1}$ lisinopril were prepared in the buffer solutions of various pH values.

These solutions were placed into the 80°C oven for 72, 96 and 120 h intervals. At the end of these intervals, the solutions were cooled immediately and a 10 μL portion of each solution was injected into the HPLC column at room temperature.

RESULTS AND DISCUSSION

Reversed phase HPLC method of Sane and Coworkers (11) was used to separate lisinopril and its degradation products in the solutions. Figure 1 shows the chromatogram of the freshly prepared lisinopril solutions. Figure 2 shows the chromatograms obtained from the solutions prepared at various pH's and kept at 80°C for 120 h.

Degradation profiles of lisinopril at acidic and neutral pH's showed that this drug has two major degradation products, observed at near 10 and 13 minutes in the chromatograms.

In Table I, degradation percentages of lisinopril at various pH values are illustrated.

Figure 3 shows the relation between pH values and degradation rate constants calculated using peak areas.

As seen in Table 1, peak area of lisinopril was increased in neutral solutions. In the low pH's, degradation rate was increased, and lisinopril peak area was decreased.

In Table 2, peak areas of the major degradation products are shown. As can be seen in the table, degradation peaks became smaller and were not observed in the neutral region.

Fig. 1: HPLC Chromatogram of Freshly Prepared Lisinopril Solution in Water (Cons:75 μgml^{-1})

a) pH = 2

b) pH = 2.5

c) pH = 3

Fig 2: HPLC Chromatograms Obtained From the Solutions Prepared at Various pH's and Kept at 80°C for 120h (Cons:75 $\mu\text{g mL}^{-1}$)

d) pH = 4

e) pH = 5

f) pH = 6

Fig 2 (cont'd): HPLC Chromatograms Obtained From the Solutions Prepared at Various pH's and Kept at 80°C for 120h (Cons:75 $\mu\text{g mL}^{-1}$)

g) pH = 7

h) pH = 8

Fig 2 (cont'd): HPLC Chromatograms Obtained From the Solutions Prepared at Various pH's and Kept at 80°C for 120h (Cons:75 μgmL^{-1})

CONCLUSION

The degradation of lisinopril can be studied by a reversed phase HPLC method. The results of this work showed that lisinopril is more stable in neutral solutions than acidic medium. Since the pharmacological activity depends on the integrity of the chemical structure, the effect of the formulation matrix is vitally important in the pharmaceutical industry. Consequently, lisinopril tablet formulations with a neutral matrix would have a better stability against temperature and humidity over those formulations comprising an acidic matrix.

Table 1
Degradation Percent of Lisinopril at Various pH's.

pH	Degradation Percent (Mean $\pm$ %S)*		
	Heating Time (hour) at 80 °C		
	72	96	120
2.0	49.22 $\pm$ %1.71	59.79 $\pm$ %0.61	67.78 $\pm$ %1.33
2.5	39.31 $\pm$ %1.00	48.29 $\pm$ %0.84	56.25 $\pm$ %0.93
3.0	27.57 $\pm$ %0.81	34.63 $\pm$ %1.50	41.44 $\pm$ %1.88
4.0	18.23 $\pm$ %1.14	23.89 $\pm$ %1.57	28.79 $\pm$ %1.75
5.0	12.46 $\pm$ %1.86	16.61 $\pm$ %1.50	20.19 $\pm$ %0.68
6.0	10.55 $\pm$ %0.93	14.17 $\pm$ %1.67	17.27 $\pm$ %1.65
7.0	9.49 $\pm$ %1.43	12.78 $\pm$ %1.87	15.47 $\pm$ %1.50
8.0	8.28 $\pm$ %1.74	11.34 $\pm$ %1.89	13.72 $\pm$ %1.37

- Calculated from three determinations

Fig. 3: The Relation of Degradation Rate Constant and pH.

Table 2
Peak areas of major degradation products at various pH's.

pH	Retention Time (min)	
	10	13
2.0	0.4300×10^6	0.3665×10^6
2.5	0.3584×10^6	0.4421×10^6
3.0	0.0370×10^6	0.3980×10^6
3.5	0.0274×10^6	0.1961×10^6
4.0	not observed	0.0089×10^6
5.0	not observed	0.0088×10^6
6.0	not observed	not observed
7.0	not observed	not observed

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