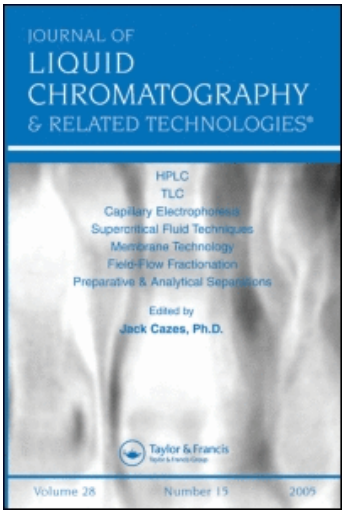

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Determination of Benazepril and Cilazapril in Pharmaceuticals by High Performance Liquid Chromatography

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DETERMINATION OF BENAZEPRIL AND CILAZAPRIL IN PHARMACEUTICALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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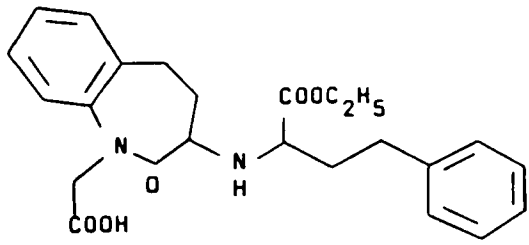
ABSTRACT

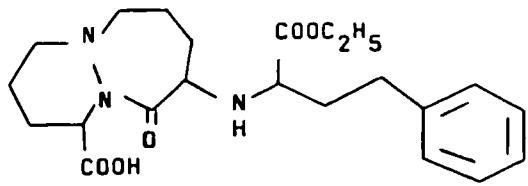
A high performance liquid chromatography method was developed for the specific determination of new angiotensin-converting enzyme inhibitors benazepril and cilazapril in pharmaceutical dosage forms. The proposed method was conducted using a reverse phase technique, UV monitoring at 211 nm and enalapril as an internal standard. The detector response was linear in the range of 10-50 μg/mL for benazepril and 40-200 μg/mL for cilazapril. The drugs were extracted from tablets with methanol. The percentage recoveries ranged from 96.34 to 102.04 and from 103.08 to 107.96 for benazepril and cilazapril, respectively.

INTRODUCTION

Benazepril-HCl, 3-[(1-ethoxycarbonyl-3-phenyl-(1S)-propyl)-amino]-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1-acetic acid hydrochloride and cilazapril, 9(S) - 1(S)-[(ethoxycarbonyl)-3-phenylpropylamino]-octahydro-10-oxo-6H-pyridazo[1,2a] [1,2]diazepine-1(S)-carboxylic acid (Figure 1), the



BENAZEPRIL



CILAZAPRIL

Figure 1. Chemical structures of investigated drugs.

angiotensin-converting enzyme inhibitors are the new drugs used in the treatment of hypertension. So far a few analytical procedures have been described for determination of benazepril, cilazapril and their active metabolites in plasma. These methods were based on gas chromatography-mass spectrometry,[1] radioimmunoassay[2] and enzymeimmunoassay.[3] Barbato et al. examined the chromatographic behaviour of several ACE inhibitors by high performance liquid chromatography.[4] The influence of different organic modifiers and of counter-ions in the mobile phase allowing the determination of the best experimental conditions for the analysis of these compounds, was investigated. However, there are no publications concerning the analysis of benazepril and cilazapril in their dosage forms. So, we decided to work out an assay procedure which would serve as a rapid and reliable method for the quality control of benazepril and cilazapril in pharmaceutical formulations.

MATERIALS

Reagents

Benazepril hydrochloride was received from Ciba-Geigy, Ltd. (Switzerland), cilazapril was received from Hoffmann-La Roche, Ltd. (Switzerland). Enalapril maleate was obtained from Polpharma S.A.(Poland). Tablets of Lotensin® (20 mg) from Ciba-Geigy and tablets of Inhibace® (5 mg) from Hoffmann-La Roche were used. HPLC grade acetonitrile and methanol were purchased from E.Merck (Germany). All the other reagents were of analytical grade.

The water needed in the experiments was double distilled. The buffer was prepared by adding phosphoric acid to 0.067 M potassium dihydrogen phosphate to obtain a final pH of 2.4.

Apparatus

The HPLC system consisted of a Model 302 solvent delivery pump from Techma-Robot Warsaw (Poland) and a Model LCD-2040 variable wavelength UV detector from Laboratorni Pristroje Praha (Czech Republic). Chromatograms were recorded with a Model TZ-4620 recorder from Laboratorni Pristroje Praha. A Model 327 reciprocating shaker from Premed (Poland) was applied.

METHODS

Chromatographic Conditions

Chromatography was carried out on LiChrosorb RP-18 column (250×4 mm) with particle size of 10 μm. The mobile phase consisted of phosphate buffer pH 2.4 and acetonitrile (7:3, v/v). The flow rate was 1 mL/min. The column effluent was monitored at 211 nm using a detector range of 0.08 AUFS and a chart speed of 0.06 cm/min. Sample volumes of 20 μL were injected into the analytical column with a manual HPLC injector fitted with a 20 μL loop (from Laboratorni Pristroje Praha). All assays were performed at ambient temperature.

Solutions

The stock solutions of benazepril-HCl (1 mg/mL), cilazapril (1 mg/mL) and of internal standard-enalapril maleate (4 mg/mL) were prepared by dissolving appropriate amounts of the substances in methanol. These solutions were stable for at least two months if stored at 4°C.

Calibration for Benazepril Assay

0.1, 0.2, 0.3, 0.4, 0.5 mL volumes of the stock solution of benazepril-HCl were pipetted into 10 mL volumetric flasks. Then, 0.2 mL volume of the internal standard solution was added to each sample and made with methanol up to the mark. 20 μl volume of each sample was then injected into the column. All measurements were repeated three times for each concentration.

The peak heights were measured and the peak height ratios of analyte to internal standard were then plotted against the respective concentration of benazepril-HCl.

Calibration for Cilazapril Assay

0.4, 0.8, 1.2, 1.6, 2.0 mL volumes of the stock solution of cilazapril and 0.5 mL volumes of the stock solution of internal standard were mixed in volumetric flasks and completed to 10 mL with methanol. A 20 μL volume of each sample was then injected into the column. All measurements were repeated three times. The calibration curve was constructed by plotting the peak height ratios of cilazapril to the internal standard versus the respective drug concentration.

Tablets of Benazepril: Extraction and Quantification

The weighed tablets of Lotensin® were ground to a fine powder. The amounts equivalent to 25 mg of the compound were extracted with methanol in 25 mL volumetric flasks. 0.1, 0.3 and 0.5 mL volumes of the filtered extracts were transferred into 10 mL flasks; 0.2 mL volumes of the internal standard solution were added and made up with methanol. Then, 20 μl volume of each sample was injected into the column.

Tablets of Cilazapril: Extraction and Quantification

Tablets of Inhibace® were weighed and pulverized. The amounts equivalent to 25 mg of the compound were extracted with methanol in 25 mL volumetric flasks. 0.4, 1.2, 2.0 mL volumes of the filtered extracts and 0.5 mL volumes of the internal standard solution were pipetted into 10 mL flasks and completed with methanol. 20 μl volume of each sample was then injected into the column.

RESULTS AND DISCUSSION

A reversed phase HPLC procedure was proposed as a suitable method for the analysis of benazepril-HCl and cilazapril in the dosage forms. The chromatographic conditions were adjusted in order to provide a versatile HPLC procedure capable of separating benazepril or cilazapril and the internal standard. A mixture of phosphate buffer, pH 2.4 - acetonitrile (7:3, v/v) at a flow rate of 1 mL/min, was found to be an appropriate mobile phase allowing adequate separation of active substances and the internal standard. As shown in Figure 2, the substances were eluted, forming well shaped, symmetrical single peaks, well separated from the solvent front.

The relationship between the peak height ratios of benazepril-HCl to the internal standard and the concentration of drug, was linear over the concentration 10-50 μg/mL. The detection limit for the analysis of benazepril was 5 μg/mL with 20 μl injection. The regression equation was y=1.46x-0.004 (standard error of slope=0.0191485, standard error of intercept=0.0127017), where y=peak height ratio of drug to that of internal standard and x=concentration of drug in μg/20 μl. The correlation coefficient for the regression line was 0.9997.

For the quantitative determination of cilazapril, the linear calibration curve was obtained in the range of 40-200 μg/mL, with the detection limit 10 μg/mL. The regression equation was y=0.31375x-0.003 (standard error of slope=0.00314576, standard error of intercept=0.0083466), where y=peak height ratio of cilazapril to that of the internal standard and x=concentration of drug in μg per 20 μl; the correlation coefficient was 0.9998.

Methanol was chosen as the extraction organic solvent because of solubility properties of the examined drugs and a reversed phase mode of chromatography. The recoveries, after extraction from the tablets, were found

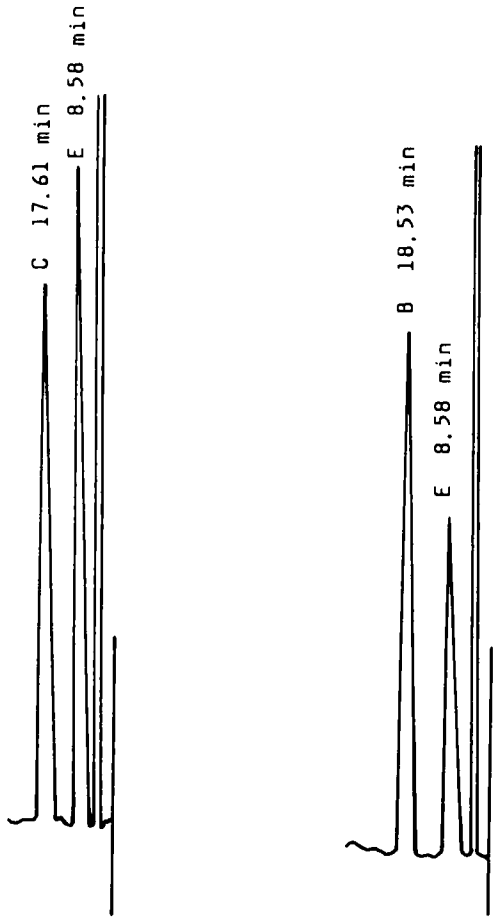


Figure 2. Typical chromatograms of benazepril (B), cilazapril (C) and internal standard-enalapril (E).

to be 99.99 ± 1.96% for benazepril-HCl and 106.04 ± 1.78% for cilazapril(mean ± standard deviation). The precision of the chromatographic analysis in tablets was determined at three concentrations of both drugs. The coefficients of variation were obtained by repeating the procedure five times for each sample (Table 1).

Table 1

Results of the Determination of Benazepril and Cilazapril in Tablets (n = 5 for each Sample)

No	Amount Expected μg/20 μL	Amount Found (Mean ± SD)	Coefficient of Variation (%)
	Benazepril		
1	0.20	0.200 ± 0.0089	4.45
2	0.60	0.584 ± 0.0102	1.75
3	1.00	1.002 ± 0.0103	1.02
	Cilazapril		
1	0.80	0.806 ± 0.0282	3.50
2	2.40	2.526 ± 0.0102	0.40
3	4.00	3.975 ± 0.0157	0.39

SD = Standard Deviation

The described method is simple and fairly reliable for the pharmaceutical analysis. As mentioned above, the literature relating to benazepril and cilazapril determinations is rather scarce. Therefore, it should facilitate the analytical investigation of both drugs.

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