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DETERMINATION OF CILAZAPRIL AND CILAZAPRILAT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRA-VIOLET DETECTION: APPLICATION TO BIOEQUIVALENCE STUDY

ODREĐIVANJE CILAZAPRILA I CILAZAPRILATA METODOM TEČNE HROMATOGRAFIJE SA ULTRALJUBIČASTOM DETEKCIJOM: PRIMENA U ISPITIVANJU BIOEKVIVALENTNOSTI

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cilazapril, cilazaprilat,
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Ključne reči
cilazapril, cilazaprilat,
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Abstract

Cilazapril (C) is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and congestive heart failure. In the liver it is metabolized into active metabolite cilazaprilat (CL). In this article, high-performance-liquid chromatographic method with ultra violet detection (HPLC-UV) for determination of cilazapril and cilazaprilat in human plasma is described. Plasma samples were prepared by solid-phase extraction. Cilazapril and cilazaprilat were separated from compounds of matrix on RP Select B column with mobile phase water-tetrahydrofuran-triethylamine (750:200:10) pH 2.5. Retention times for C and CL were 5.9 and 13.9 min. Method was linear in the concentration range of 5-200 ng/mL. HPLC-UV method was applied in the pharmacokinetic study of generic tablets with the new formulation, containing cilazapril, after single oral dose of 2.5 mg that was given to 12 healthy volunteers.

INTRODUCTION

Cilazapril (C) is a specific, long-acting angiotensin-converting enzyme (ACE) inhibitor which suppresses the renin-angiotensin-aldosterone system and thereby the conversion of the inactive angiotensin I to angiotensin II, which is a potent vasoconstrictor.

It induces a reduction of blood pressure. The antihypertensive effect of cilazapril is usually apparent within the first hour after administration, with maximum effect observed between 3 and 7 hours after dosing.

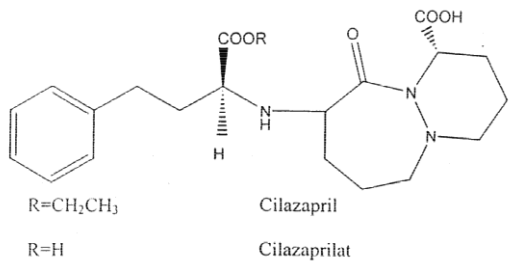

Figure 1. Molecular structure of cilazapril and cilazaprilat

Cilazapril is efficiently absorbed and rapidly converted to the active form, cilazaprilat (CL). Maximum plasma concentrations are reached within 2 hours after administration and are directly related to dosage.

Doses of cilazapril range from 0.5 to 5 mg per day. It is eliminated unchanged by the kidneys, with an effective half-life of 9 hours after once-daily dosing with cilazapril. The elimination kinetic of cilazaprilat is biphasic. The distribution half-life is 1.5-2h and the terminal elimination half-life is 30-50h [1-4].

Many formulations of cilazapril are available on the Serbian market today[5]. There are not many literature data about determination of cilazapril and cilazaprilat in biological samples. Efficacy of these compounds has been usually determined by measuring ACE inhibition, angiotensin II or rennin levels[6-7]. The methods for determination of cilazapril in pharmaceuticals and biological samples use high performance liquid chromatography (HPLC) with photometric, amperometric, ultraviolet or mass spectrometric detection [8-13].

We describe HPLC-UV method determination of clazapril and cilazaprilat in plasma and its application to pharmacokinetic study.

MATERIAL AND METHODS

Analytical standard of cilazapril (99.5%) and cilazaprilat (99.45%) obtained by Ranbaxy, India. Tetrahydrofurane, triethylamine, orto-phosphoric acid, methanol, dichloromethane, and hydrochloric acid were of HPLC and p.a. purity, obtained from Merck.

Chromatographic conditions

Chromatographic system consisted of LKB Bromma 2150 HPLC Pump connected to BioRad Model 1801 UV Monitor which operated at 220 nm. Separation of cilazapril and cilazaprilat from matrix compound was performed on Lichrospher RP select B 60, 250-4, Merck, with guard column Lichrochart 4-4 RP Select B at room temperature. The mobile phase consisted of triethylamine:water (10:750) pH 2.5 (adjusted with orto-phosphoric acid) and 200 mL of tetrahydrofurane. Flow of mobile phase was 1,0 mL/min. Volume of 200 µL samples were injected by automatic sampling System BioRad Model AS-100 HPLC. Retention times for C and CL were 5.9 and 13.9 min.

Sample preparation

Samples were prepared by solid phase extraction on Oasis HLB cartridges (Waters).

After melting at room temperature in 1 mL of plasma sample 0,1 mL 1M HCl was added. Homogenised acid plasma sample passed through a cartridge previously conditioned with 1 mL of methanol and 1 mL of demineralized water. HLB cartridge then was washed with 1 mL of 5 % methanol and C and CL were eluted with 4 mL of dichloromethane. Eluat was evaporated to dryness, reconstituted in mobile phase and analyzed by HPLC-UV method.

Calibration and quality control samples were prepared by adding of C and CL solution in blank („drug-free") human plasma. The amounts corresponded to plasma concentration of C and CL ranging from 5 ng/mL to 200 ng/mL. The calibration curves for plasma spiked by C and CL were obtained by plotting their peak areas for the concentrations range of 5, 20, 50, 80, 100 and 200 ng/mL.

RESULTS

Concentrations of C and CL were determined using weighed linear regression function. Figures 2. and 3. show calibration curve for plasma spiked by C and CL.

Recovery of the extraction for C and CL from plasma ranges from 94.71-101.25 % (mean 97.91 %) and 96.21-100.34 % (mean 98.13 %), respectively.

Figure 4. shows chromatogram of plasma spiked by C and CL solution concentration of 20 ng/mL.

Table 1. shows inter and intra day coefficient of variation (CV) for cilazapril and cilazaprilat

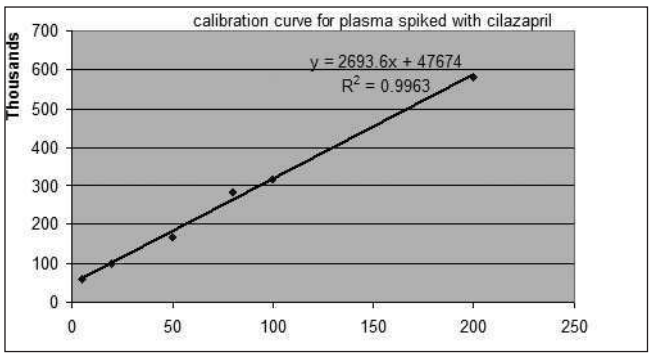

Figure 2. *Calibration curve for plasma spiked by cilazapril*

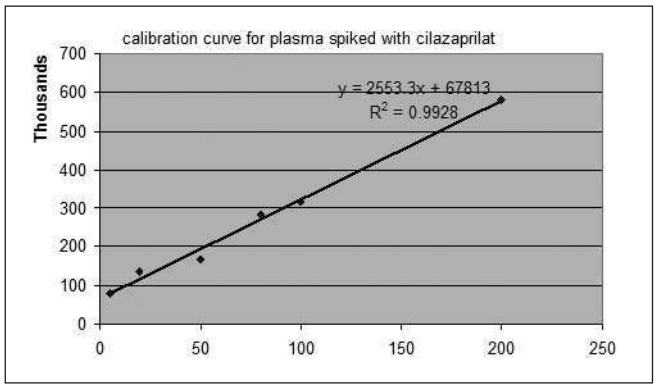

Figure 3. *Calibration curve for plasma spiked by cilazaprilat*

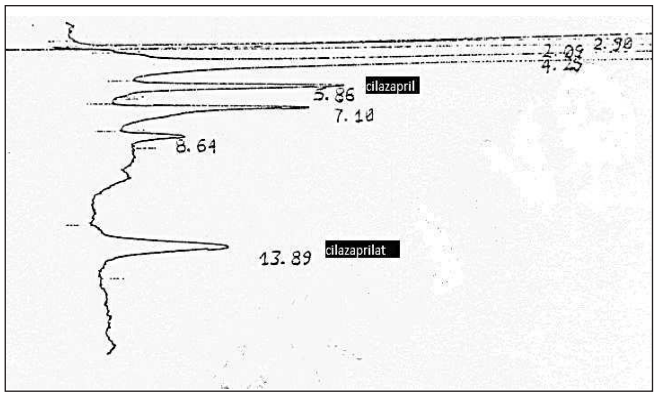

Figure 4. *Chromatogram of plasma spiked by cilazapril and cilazaprilat*

Table 1. *Inter-day and intra-day precision.*

concentration ng/mL	inter-day CV (%) cilazapril	intra-day CV (%) cilazapril	inter-day CV (%) cilazaprilat	intra-day CV (%) cilazaprilat
5	4.79	5.69	7.75	6.37
50	3.07	4.09	3.62	5.03
200	2.87	2.91	3.13	3.01

Table 2. *Stability of C and CL after freezing and thawing (F/T)*

conc. of C and CL (ng/mL)	conc. of C after F/T (ng/mL)	recovery (%)	conc. of CL after F/T (ng/mL)	recovery (%)
5	4.78	95.60	4.86	97.20
50	52.36	104.72	49.12	98.24
200	192.74	96.37	195.03	97.51

Cilazapril and cilazaprilat concentrations down to 5 ng/mL can be accurately and precisely measured using 1 mL of plasma sample. (Table 2)

Cilazapril and cilazaprilat in plasma were stable following 3-fold freezing and thawing procedure.

The bioequivalence study of two cilazapril tablet formulations was assessed in 12 healthy volunteers who received a single 2.5 mg dose of each cilazapril formulation. Dosing in each of the two consecutive periods was separated by a 2-week wash-out period. Venous blood samples were collected prior to dosing (time 0) and afterwards at time-points 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12 and 24 hours. Tables 3. and 4. show mean values of pharmacokinetic parameters for C and CL

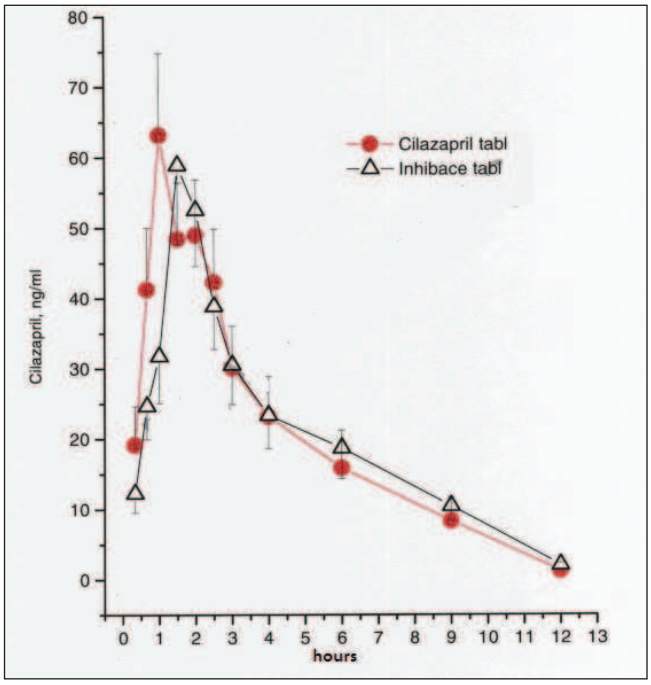

Figure 5. *Mean (± SD) log-concentration vs time profiles of cilazapril in the serum after the peroral administration of a 2.5 rng cilazapril or Inhibace tablet to 12 healthy volunteers.*

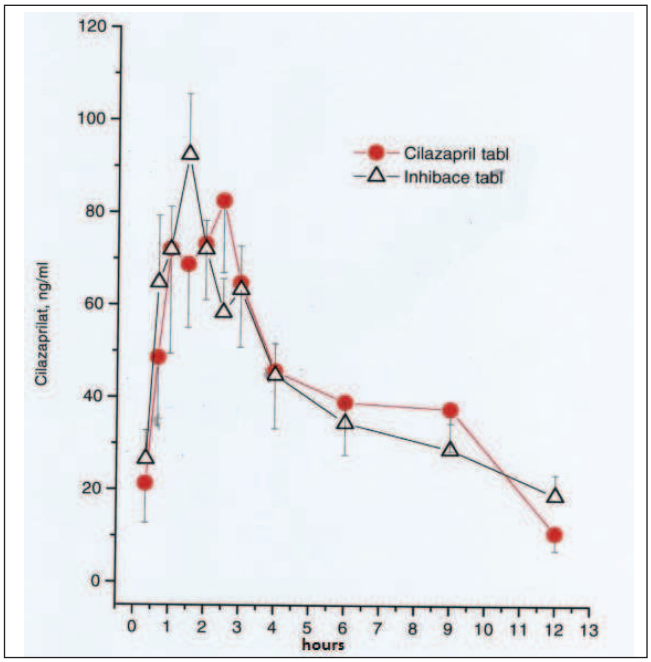

Figure 6. *Mean (± SD) log-concentration vs time profiles of cilazaprilat in the serum after the peroral administration of a 2.5 rng cilazapril or Inhibace tablet to 12 healthy volunteers.*

parameter	Inhibace mean value	Cilazapril mean value	ratio (%)
C_{max} (ng/mL)	69.54	76.57	110.11
t_{max} (h)	1.79	1.54	86.03
$t_{1/2}$ (h)	3.00	2.87	95.67
AUC^{0-24} (ng*h/mL)	255.59	230.61	90.23
$AUC^{0-\infty}$ (ng*h/mL)	286.94	265.96	92.69

Table 3. *Mean values of pharmacokinetic parameters of cilazapril netic parameters of cilazaprilat after administration of Cilazapril and Inhibace tablets dose 2.5 mg*

parameter	Inhibace mean value	Cilazapril mean value	ratio (%)
C_{max} (ng/mL)	119.66	121.01	101.13
t_{max} (h)	1.46	2.14	146.58
$t_{1/2}$ (h)	7.01	6.89	98.29
AUC^{0-24} (ng*h/mL)	480.17	515.38	107.33
$AUC^{0-\infty}$ (ng*h/mL)	733.72	786.09	107.14

Table 4*. Mean values of pharmacokinetic parameters of cilazaprilat after administration of Cilazapril and Inhibace tablets dose 2.5 mg*

Mean serum concentration-time profiles of cilazapril and its metabolite cilazaprilat are shown in Figures 5. and 6.

DISSCUSION

According to the literature separation of cilazapril and cilazaprilat can be done on C18 column with metanol-10mM phosporic acid (50:50) mobile phase, at flow rate of 1 mL/min, and UV detection on 206 nm [12]. Described chromatographic condition were not suitable for determination of C and CL in serum samples after applying single dose of 2.5 mg of cilazapril, because there is matrix interference on 206 nm. We changed mobile phase, wavelength and type of column. Applying of the mobile phase consisted of triethylamine:water (10:750) pH 2.5 (adjusted with orto-phosphoric acid) and 200 mL of tetrahydrofurane on select B column, with detection on 220 nm gave good separation of C and CL from matrix compound. The pH of mobile phase was similar to pH values described in literature [11-12].

Since the detection performed at a low wavelength, liquid-liquid extraction did not give good results because of interferences of endogenous serum compounds. We chose solid-phase extraction (SPE) to get cleaner extracts with better recovery. We used Oasis HLB cartridges for preparation of serum samples. Prior to SPE we acidified samples to get better recovery. The mean recoveries of the extraction for C and CL from plasma were 97.91 % and 98.13 %, respectively, which were better than recovery of 85% on C8 cartridges [8,12].

The method was linear in the concentration range of 5 to 200 ng/mL, which was in the expected therapeutic range. Limits of quantitation for C and CL of 3.4 and 2.8 ng/mL were respectively satisfactory.

As expected, described HPLC-UV method was less sensitive than liquid chromatography with mass spectrometry [14-15]. Despite the fact, analysis of assay data indicated that HPLC-UV method was simple, precise and accurate enough for performing bioequivalence study.

Application to bioequivalence study

We applied described HPLC-UV method for determination of C and CL in bioequivalence study. A single-dose, open-label, randomized, two-sequence, two period crossover study design was used to evaluate the bioavailability of cilazapril prepared by two different manufacturers as tablets. Figure 5 and 6 show the mean serum concentration–time curve for cilazapril and cilazaprilat.

Standard statistical methods were used to analyze relative differences between the drugs.

The respective point estimates of ratios of the geometric means of long-transformed cmax and $AUC_{0-\infty}$ of cilazapril (test-reference) were 98.05 and 103.31, with 90% CIs of 92.73-104.54 and 99.94-107.08, respectively.

The respective point estimates of ratios of the geometric means of long-transformed cmax and $AUC_{0-\infty}$ of cilazaprilat (test-reference) were 104.59 and 106.21, with 90% CIs of 98.74-111.89 and 100.19-113.64, respectively.

Mean maximal cilazapril serum concentrations, were 119.66 ng/mL (reference) and 121.01 ng/mL (test). The half-life was 6.89 h. The area under the serum concentration–time curve (AUC_{0-24}) was 230.61 ng.h/mL.

Mean maximal cilazaprilat serum concentrations, were 69.54 ng/mL (reference) and 76.57 ng/mL (test). The half-life was 2.87 ± 1.07 h. The area under the serum concentration–time curve (AUC_{0-24}) was 515.381 ng.h/mL.

No adverse effects were reported by the subjects or revealed by clinical or laboratory tests.

The overall pharmacokinetic profile of cilazapril and cilazaprilat in the present study was close and in agreement with the data previously published for cilazapril preparations in the relevant publications [16-17].

Statistical data show that test drug cilazapril tablets were considered bioequivalent to the reference Inhibace tablets.

CONCLUSION

Described HPLC-UV method for the determination of cilazapril and cilazaprilat is simple, selective, precise and suitable for bioequivalence study. From the pharmacokinetic point of view both of the studied drugs have to show therapeutic equivalence in patients. There were no differences in the bioequivalence assessed either for cilazapril or its metabolite cilazaprilat.

Sažetak

Cilazapril (C) je inhibitor angiotenzin-konvertujućeg enzima koji se koristi u lečenju hipertenzije i kongestivnog oboljenja srca. U jetri se metaboliše u aktivni metabolit cilazaprilat (CL). U radu je opisana metoda visokoefikasne tečne hromatografije sa ultraljubičastom detekcijom (HPLC-UV) za određivanje cilazaprila i cilazaprilata u humanoj plazmi. Uzorci plazme pripremani su čvrsto-faznom ekstrakcijom. Cilazapril i cilazaprilat su na RP Select B koloni odvojeni od komonenata matriksa korišćenjem mobilne faze voda-tetrahidrofuran-trietilamin (750:200:10) pH 2.5. Retenciona vremena cilazaprila i cilazaprilata bila su 5,9 i 13,9 min. Metoda je bila linearna u opsegu koncentracija od 5-200 ng/mL. HPLC-UV metoda je primenjena u farmakokinetičkom ispitivanju tableta koje sadrže cilazapril nakon primene pojedinačne oralne doze od 2,5 mg koja je data zdravim dobrovoljcima.

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