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Simultaneous HPLC determination of Enalapril and Hydrochlorothiazide in Human Plasma and its pharmacokinetic application

Nagwa H . Foda* ,O. Naeem** , A . Abd ELbary **and G. Abd ELbary**

* king Abdulaziz university, Faculty of Pharmacy, KSA

**Center of applied research and Advanced studies (CARAS), Faculty of Pharmacy, Cairo university.

Abstract

A sensitive and specific HPLC method has been developed and validated for the simultaneous determination of enalapril and hydrochlorothiazide in human plasma using C18 reversed-phase column.The average recoveries range from 0.005-0.1µg/ml and 0.01-0.2 µg/ml for each drug were 92.7% and 93.3 % respectively. The limit of detection were 2.5 and 0.14 ng /ml for enalapril and hydrochlorothiazide respectively. the validated method has been used successfully to study enalapril and hydrochlorothiazide pharmacokinetic, bioavailability and bioequivalence in 24 adult volunteers.

Keywords: *Enalapril Hydrochlorothiazide: HPLC determination; human plasma; bioequivalence; pharmacokinetics; bioavailability.*

INTRODUCTION

Enalapril maleate [(S)-1-(N-(1-(Ethoxycarbonyl)-3-phenylpropyl)-L-alanyl)-L-proline (Z)-2-butenedioate salt] is an angiotensin-converting enzyme (ACE) inhibitor, used for oral treatment of hypertension. It blocks the ACE mediated conversion of angiotensin I to Ang II and the inactivation of kinins, leading to decreased levels of vasoconstrictor peptide AngII and accumulation of kinins (Unger and Stoppelhaar, 2007). This agent is able to reduce cardiovascular mortality and morbidity in patients with heart failure (Santos et al., 2009).

Hydrochlorothiazide [6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide] is used as a diuretic. Diuretics, in particular hydrochlorothiazide (HCTZ), are often avoided as monotherapy in the management of hypertension in patients with ischemic heart diseases (Asqada and Inamda, 2009). Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride in approximately equivalent amounts. Indirectly, the diuretic action of hydrochlorothiazide reduces plasma volume, with consequent increase in urinary potassium loss, plasma renin activity, aldosterone secretion and decrease in serum potassium (Field et al., 1984). In patients with cardiac ischemia, heart failure or left ventricular hypertrophy, even mild-to-moderate hypokalemia increases the likelihood of cardiac arrhythmias (Hoes et al., 1994). The combination of HCTZ with ACE-I, aldosterone antagonist or angiotensin II type 1 receptor blocker is found to minimize the potassium loss characteristically induced by the thiazide component.

Few methods have been reported for the simultaneous determination of enalapril and hydrochlorothiazide both in-vitro and in-vivo. The two drugs were determined simultaneously in pharmaceutical formulations using high performance liquid chromatographic methods (HPLC), first-derivative (el Walily et al., 1995) and second-derivative (Carlucci et al., 1993) ultraviolet spectra methods. A HPLC method was developed for the determination of the two drugs in tablets using ultraviolet (UV) detection (Al-Momani, 2001). A chemometric approach using HPLC with photodiode array detection was also developed and applied for the simultaneous determination of the two drugs in tablets (Erdal et al., 2005).

A selective and sensitive HPLC method with mass spectrometry detection (LC-MS) was applied for the simultaneous determination of enalapril, enalaprilat and hydrochlorothiazide in plasma samples in a bioequivalence study of two immediate release tablets of enalapril/hydrochlorothiazide in healthy volunteers (Maya et al., 2002).

The proposed method is of comparable sensitivity to the published LC-MS methods (Maya et al., 2002). It also has the advantage of being more economic, as it uses UV detection.

EXPERIMENTAL

Chemicals and Reagents

Enalapril And Hydrochlorothiazide were supplied by (ACAPI) chemical Co. Cairo Egypt, Caffeine anhydrous was supplied by (Kuwait Saudi- pharmaceutical industries,K.S.A). Methanol and Acetonitrile (HPLC grade) were supplied by (Romil Limited, London, United Kingdom & E. Merk , D-6100 Darmstadt, F.R. Germany). Water used was HPLC grade.

Instrumentation

A Shimadzu HPLC system was utilized consisting of the following components: isocratic pump LC – 10 AS, degasser unit DGU-12A and a UV /VIS variable detector SPD – 10 A., an integrator C-R 6A.Chromatographic separation was accomplished using C18 reversed-phase column, waters Associate 3.9 i.d x 150mm.

Chromatographic conditions

Mobile phase: phosphate buffer: Acetontrile (80: 20) v/v pH 3.4 adjusted with orthophosphoric acid . The flow rate was 1ml/min, the detection wavelength 265 nm and the sensitivity was set at 0.0001 AUFS. All assays were performed at ambient conditions.

Standard Solution of enalapril and hydrochlorothiazide

A stock solution of each of enalapril or Hydrochlorothiazide were prepared by dissolving 10 mg of each drug separately In 100ml of methanol. The working standard solution was prepared by taking 10ml from each of the above solution in 100 ml methanol (10 µg/ ml for each drug).

Internal Standard Solution

A stock internal standard solution was prepared by dissolving 10 mg of Caffeine anhydrous in 100ml methanol. The working internal standard was prepared by taking 10 ml from this solution in 100 ml methanol (10µg/ml).

Calibration Curve

Standard samples were prepared by transferring aliquots of working standard solutions at concentrations of enalapril and hydrochlorothiazide ranging from (0.005 – 0.1) µg/ml and (0.01-0.2) µg/ml respectively into centrifuge tubes provided with tight sealing polyethylene caps, containing 1ml plasma .

1 ml of the working internal standard in methanol was added to each tube.

Extraction by Precipitation Procedure

1 ml acetonitrile was added to each sample vortexed for 20 seconds and centrifuged for 10 minutes at 3000 r.p.m. The upper layer was transferred to another tube filtered through 0.45 µm millipore filter, evaporated by nitrogen at ambient temperature, then reconstituted with 100 µl mobile phase . 20 µl were injected on the column for analysis.

Under the conditions described, the retention time of enalapril, hydrochlorothiazide, and Caffeine anhydrous were3.49, 7.5and 5.66min respectively.

Quatitation

The calibration curves of the peak area ratio of enalapril to Caffeine anhydrous and hydrochlorothiazide to Caffeine anhydrous versus enalapril and hydrochlorothiazide concentration respectively were done. 1ml of the different plasma samples obtained from the volunteers were treated as mentioned. The unknown sample concentration was calculated from the following formula:

$$Q = \{R/A + B\} \times \text{dilution factor}$$

Where Q is enalapril or hydrochlorothiazide concentration, R is the peak area ratio (drug/internal standard), A is the slope of the calibration curve and B is the Y-intercept.

BIOEQUIVALENCE STUDY

Subject

Twenty-four healthy male volunteers with a mean age of 24.2 1 years, a mean height of 170.42 cm and a mean weigh of 72.0 kg participated in this study. The volunteers underwent physical examination and complete hematological and biochemical examinations. None of the volunteers had any history of drug or alcohol abuse, nor did they have any acute or chronic gastrointestinal, cardiac, vascular, hepatic or renal disease. No concurrent

medication was allowed during the course of the study.

Study Design

This is randomized, 2-way crossover design study. The study was performed in two phases: In phase I half the number of the volunteers received one tablet(10/ 12.5mg) of product B and the remainder received one tablet(20 /12.5 mg) of product A which is taken as standard.

A washout period of 1 week separated the two phases, and in the second phase, reverse of randomization occurred so that the volunteers who received treatment A in the first phase received treatment B on the second phase and vice versa. Each group was supervised by a physician who was responsible for their safety collection of samples during the trial and supervised each group. A supervised fast of at least 12-hour duration began with the completion of dinner and lasted until the administration of the dose. At the beginning of the study, all the volunteers were cannulated and a control sample of 5 ml blood was withdrawn before drug administration.

Sample Collection

Samples were obtained at 0.0, 0.50**,** 1.0**,** 1.5**,** 2**,** 3**,** 4**,** 5**,** 6and 24 h post dose .All the samples were collected and immediately centrifuged and the plasma was frozen and stored at -20°C until the analysis.

Analysis of Plasma Samples

The aforementioned HPLC assay was used.

Pharmacokinetic Calculation

Pharmacokinetic parameters determined for enalapril and hydrochlorothiazide plasma concentration-time data are:

1. **C max**: is the highest drug concentration during the 24-hour study period.
2. **T max**: is the time taken to reach C max.
3. **AUC (0-t)**: is the area under the plasma concentration time curve from time zero to 24- hours.
4. **AUC (0-∞)** : is the area under the plasma concentration time curve from time zero to infinity.
5. **t ½** : is the half life of elimination of enalapril and hydrochlorothiazide.
6. **K**: is the elimination rate constant of enalapril and hydrochlorothiazide.

RESULTS AND DISCUSSION

HPLC Assay

Chromatograms obtained at the lower limit of sensitivity for drug free plasma extracts showed no interfering peaks at the retention times of enalapril , hydrochlorothiazide and the internal standard. Figure 1 shows a typical chromatogram for the samples prepared from blank human plasma containing enalapril, hydrochlorothiazide and caffeine anhydrous. Using the chromatographic conditions described, enalapril ,hydrochlorothiazide and caffeine anhydrous were well Separated and their retention times were 3.49, 7.5and 5.66 min, respectively. All peaks were sharp and symmetrical with good baseline resolution(R= & R =) and minimal tailings (tailing factor = 1), (capacity factor K` = &) and selectivity (a) =1.39 &1.5) Accordingly ,the selected conditions show an optimum detection and determination of enalapril and hydrochlorothiazide. Caffeine anhydrous is a good internal standard because of its adequate retention time and similar spectral properties to enalapril and hydrochlorothiazide. No interferences by the metabolites or normal constituents of plasma were observed.

Plasma samples spiked with different amounts of enalapril and hydrochlorothiazide were analyzed 3 times on the same day. Table1 (A& B) Show the standard plots obtained for enalapril and Hydrochlorothiazide in plasma samples. Linear regression analysis of the standard calibration plot for human plasma were y1= 0.0102 x1 + 0.0000627 and y2= 0.082 x2 + 0.000114 where y1, x1 and y2, x2 are the peak area ratio and enalapril or hydrochlorothiazide concentration, respectively . The good linearity is evident from the value of the correlation coefficient (r > 0.9999. The RSD percent of the slope and the intercept were 0.634,11.6 for enalapril and 0.2583,3.6 for hydrochlorothiazide respectively.

The minimum detectable concentrations of enalapril and hydrochlorothiazide (LOD) (3s/m) were calculated and found to be 0.00255 µg/ml and 0.0001467 µg/ml. The LOQ (10s /m) were calculated and found to be

0.0085 µg/ml and 0.00048 µg/ml for enalapril and hydrochlorothiazide which are less than the lowest concentration in the calibration curves of enalapril (0.005-0.1) µg/ml and hydrochlorothiazide (0.01-0.2 µg/ml) .The accuracy was assessed by spiking plasma with different concentrations of enalapril (0.005-0.1)and (0.01-0.2 µg/ml) hydrochlorothiazide
The percent recovery ranged from 91-95 % and 92 - 95 % , with a with a mean percent of 92,7 %,and 93.33 %, and was not concentration dependent (Table 2 A&B). The analytical precision of the method is determined by the relative standard deviation of the percentage drug recovered(RSD% =2.87 2.73). The day – to day reproducibility of the assay for plasma samples was evaluated by comparing the least squares linear regression analysis of three standard plots for each drug obtained from spiked plasma standard at three different days over a three - weeks period. The results of these evaluations are summarized in Table 3 (A & B). Analysis of variance of the data showed no detectable difference in the slopes of the three standard plots (F =3.1 ,p>0.01) ,& (F=2.7 , p>0.01) for enalapril maleate and hydrochlorothiazide respectively.

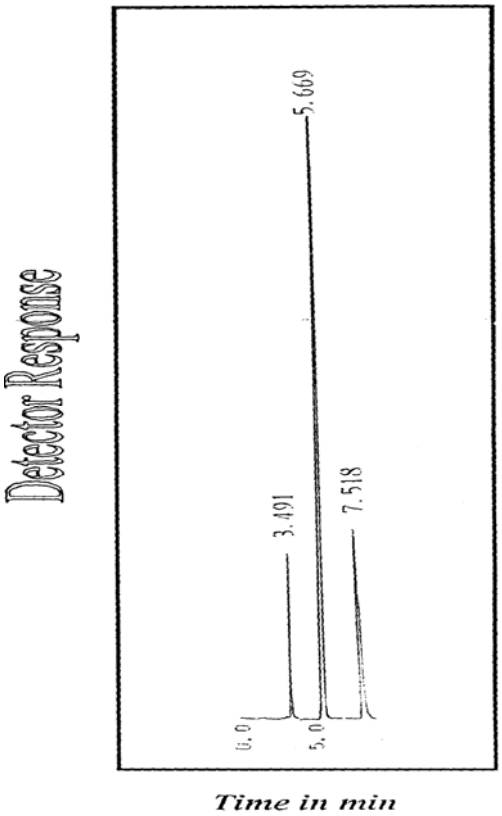

Figure 1 : Chrornatograrn of Enalapril maleate & Hydrochlorothiazide in Human Plasma Key
- Enalapril maleate 3.49 min
- Hydrochlorothiazide 7.5 min
- Caffeine anhydrous 5.66 min

Table 1(A) Analytical Precision for the Analysis of Enalapril Performed on 3 sets of Standard Curves on the Same Day

Spiked Conc. (µg/ml)	***Peak Area Ratio***			***Mean***	***SD***	***RSD%***
	1	***2***	***3***			
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.005	0.0005	0.00048	0.00047	0.00048	0.0000124	2.598
0.01	0.001	0.001	0.00099	0.000996	0.0000047	0.473
0.02	0.00201	0.00202	0.00203	0.00202	0.00000816	0.404
0.04	0.00401	0.00402	0.00402	0.00401	0.0000047	0.117
0.06	0.00602	0.00602	0.00601	0.006016	0.0000047	0.0788883
0.08	0.00802	0.00803	0.00804	0.00803	0.00000816	0.101
0.1	0.0104	0.0105	0.0106	0.0105	0.00000816	0.777
r	0.9996	0.9994	0.9991	0.9994		
Slope	0.0102	0.0`03	0.0104	0.0104		
intercept	0.0000627	-0.000083	-0.000105	-0.0000863		

RSD % of slope = 0.634 ; RSD %of intercept= 11.6 ; LOD =0.00255 ***µg*** /ml; LOQ =0.0085 ***µg*** /ml

Table 1(B) Analytical Precision For the Analysis of Hydrochlorothiazide Performed on 3 sets of Standard Curves on the Same Day

Spiked Conc. (µg/ml)	***Peak Area Ratio***			***Mean***	***SD***	***RSD%***
	1	***2***	***3***			
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.01	0.00076	0.00075	0.00074	0.00075	0.00000816	1.0886
0.02	0.00167	0.00166	0.00168	0.00167	0.00000816	0.488
0.04	0.0032	0.00318	0.00322	0.0032	0.0000163	0.51
0.06	0.0049	0.00488	0.00485	0.00487	0.0000205	0.421
0.008	0.0065	0.0064	0.00651	0.00648	0.0000659	1.018
0.1	0.0082	0.0083	0.00822	0.00824	0.0000432	0.524
0.2	0.0167	0.0166	0.0167	0.0166	0.0000471	0.284
r	0.9999	0.9998	0.9999	0.9999		
Slope	0.0837	0.0833	0.0838	0..0832		
Intercept	-0.000109	-0.000117	-0.0001145	-0.000094		

RSD % of slope =0.2583 ; RSD %of intercept=3.6 ; LOD =0.0001467 ***µg*** /ml; LOQ =0.00048 ***µg*** / ml

Table 2(A) Within day accuracy for the determination of Enalapril in plasma

a. Number of replicate samples

Enalapril (µg/ml)	***n a***	***Mean Recovery (µg/ml)***	***SD***	***Recovery b %***	***RSDc %***
0.005	3	0.0455	0.00154	91	3.4
0.01	3	0.0092	0.000322	92	3.5
0.02	3	0.0184	0.000057	92	3.1
0.04	3	0.0376	0.00109	94	2.9
0.06	3	0.055	0.00132	93	2.4
0.08	3	0.0736	0.00184	92	2.5
0.10	3	0.095	0.00218	95	2.3

b. Average recovery =92.714

c. Average RSDc % = 2.871

*Table 2(B)****Within day accuracy for the determination of Hydrochlorothiazide in plasma***

Hydrochlorothiazide *(µg/ml)*	**n a**	***Mean Recovery (µg/ml)***	**SD**	**Recovery b %**	**RSD%**
0.01	3	0.0092	0.00028	92	3.1
0.02	3	0.0188	0..000601	94	3.2
0.04	3	0.0372	0.000967	93	2.6
0.06	3	0.0552	0.00154	92	2.8
0.08	3	0.0752	0.0018	94	2.4
0.1	3	0.095	0.00218	95	2.3
0.2	3	0.19	0.00513	95	2.5

a. Number of replicate samples

b. Average recovery = 93.57

c. Average RSD%=2.7

Table 3(A) Day to Day Reproducibility Data for the Standard Plots of Enalapril in Plasma

Standard a plot	***Slope b***	***Intercept b***	***Correlation b Coefficient***
1	0.01028	-0.0000627	0.9996
2	0.01036	-0.000083	0.9994
3	0.01035	-0.000079	0.9994

a. Obtained in 3 different days.

b. The mean of 3-6 determination at each drug concentration.

Table 3(B) Day to Day Reproducibility Data for the Standard Plots of Hydrochlorothiazide in Plasma

Standard a plot	*Slope b*	*Intercept b*	*Correlation b Coefficient*
1	*0.0837*	-0.000109	0.9999
2	*0.0833*	-0.000117	0.9998
3	*0.0838*	-0.0001145	0.9999

a Obtained in 3 different days.
b The mean of 3-6 determination at each drug concentration.

BIOEQUIVALENCE STUDY:

The mean plasma concentration time data of enalapril maleate and hydrochlorothiazide following the oral administration of the two products A and B is illustrated graphically in Figure 2 A and 2 B for both tablet products. The pharmacokinetic parameters determined for both products are summarized in Table 4 and 5.

It is shown that the peak plasma concentration (C max) of enalapril maleate following the administration of product A ranged from: 0.31 to 0.371 µg/ml, with a mean value of 0.3 ± 0.086 µg/ml. The peak plasma concentration of enalapril maleate following the administration of product B ranged from: 0.310to 0.360 µg/ml, with a mean value of 0.331 ± 0.016 µg/ml. The time to peak concentration (t max) was 2.0 hour for both A and B Tablets.

The area under the plasma concentration time curve AUC (0-∞) for enalapril maleate following the administration of product A ranged from 2.05 to 2.35 µg .hr/ml, with a mean of 2.26 ± 0.07 µg .hr /ml. While the AUC(0-∞) after product B administration was in the range of 2.14 to 2.35 µg .hr /ml, with a mean of 2.23± 0.04 µg .hr/ml. The AUC(0-t) of enalapril maleate following the administration of product A ranged from: 1,35to 1,65 µg .hr /ml, with a mean value of 1.58± 0.07µg .hr /ml. Whereas, AUC(0-t) of enalapril maleate following the administration of product B ranged from: 1.50 to 1.58µg .h /ml, with a mean value of 1.53 ± 0.02 The t 1/2 of elimination of enalapril maleate following the administration of product A ranged from: 8.38 to 4.08hours, with a mean value of 6.19± 1.03 hours. Whereas, t 1/2 of elimination of enalapril maleate following the administration of product B ranged from: 10.87- to 2.21 hours, with a mean value of 5.27± 2.21 hour. The elimination rate constant K of enalapril maleate following the administration of product A ranged from: 0.17to 0.08 hour-1, with a mean value of 0.12± 0.02 hour-1. The elimination rate constant of enalapril maleate following the administration of product B Tablets ranged from: 0.31to 0.06 hour-1, with a mean value of 0.16 ± 0.07 hour –1.The percentage relative bioavailability of enalapril maleate from product B Tablets compared to product A Tablets was found to be 98.60 % as determined by the ratios between the AUC (0-∞) of product B to product A .

The peak plasma concentration (C max) of hydrochlorothiazide following the administration of product A ranged from: 0.054 to 0.059 µg/ml, with a mean value of 0.057 ± 0.002 µg/ml.The peak plasma concentration of hydrochlorothiazide following the administration of product B ranged from: 0.054 to 0.059µg/ml, with a mean value of 0.057 ± 0.002 µg/ml.

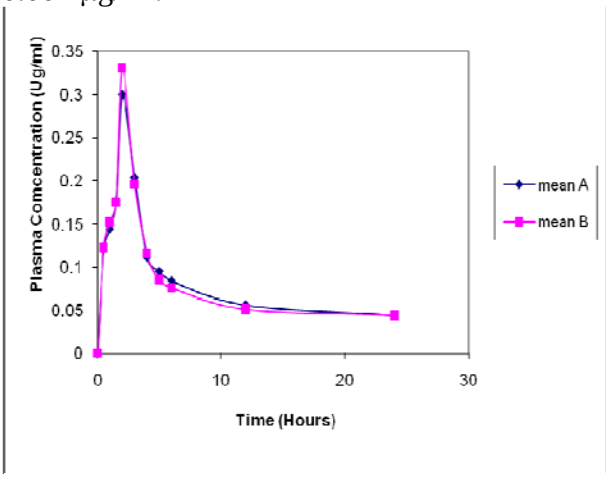

Fig (2A) Mean Plasma Concentration Time Curve of Enalapril maleate following the Administration of product A &B tablets

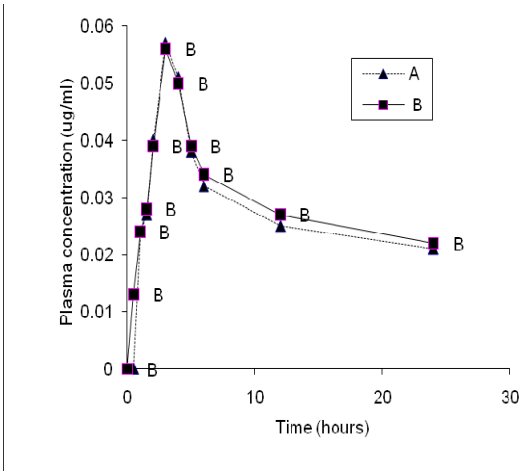

Fig (2B) Mean Plasma Concentration Time Curve of Hydrochlorothiazide following the Administration of product A &B tablets

Table (4): Pharmacokinetic Parameters of Enalapril Following the Oral Administration of Product A.

Subject	*Cmax (µg /ml)*	*t ½ (hr)*	*K (hr-1)*	*tmax (hr)*	*AUC (0-t) µg.hr/ ml*	*AUC (0-∞) µg.hr/ ml*
1	0.303	4.7	0.15	2.0	1.58	2.20
2	0.305	5.1	0.13	2.0	1.58	2.27
3	0.304	7.1	0.10	2.0	1.58	2.26
4	0.325	5.3	0.13	2.0	1.58	2.29
5	0.305	7.2	0.10	2.0	1.57	2.23
6	0.304	5.6	0.12	2.0	1.61	2.29
7	0.336	5.0	0.14	2.0	1.63	2.33
8	0.305	4.0	0.17	2.0	1.60	2.3
9	0.319	7.2	0.10	2.0	1.59	2.28
10	0.301	5.3	0.13	2.0	1.35	2.05
11	0.304	5.6	0.12	2.0	1.52	2.22
12	0.371	6.9	0.10	2.0	1.63	2.31
13	0.303	6.4	0.11	2.0	1.54	2.19
14	0.305	6.3	0.11	2.0	1.55	2.22
15	0.302	6.9	0.10	2.0	1.37	2.08
16	0.342	5.7	0.12	2.0	1.59	2.26
17	0.364	6.4	0.11	2.0	1.62	2.30
18	0.304	7.0	0.10	2.0	1.59	2.30
19	0.354	5.7	0.12	2.0	1.62	2.31
20	0.367	6.9	0.10	2.0	1.63	2.34
21	0.310	8.3	0.08	2.0	1.6	2.28
22	0.341	6.4	0.11	2.0	1.65	2.35
23	0.361	7.1	0.10	2.0	1.65	2.35
24	0.320	5.1	0.14	2.0	1.58	2.27
Mean	**0.300**	**6.1**	**0.12**	**2.0**	**1.58**	**2.26**
± S.D	**0.086**	**1.0**	**0.02**	**0.00**	**0.07**	**0.07**
Range	**0.31-0.371**	**8.8-4.0**	**0.17-0.18**	**2.0-2.0**	**1.35-1.65**	**2.05-2.35**
Median				**2.0**		

The time to peak concentration (t max) was 3 hours for both A and B Tablets. The area under the plasma concentration time curve AUC (0-∞) for hydrochlorothiazide following the administration of product A ranged from 0.79- to 0.96 µg .hr /ml, with a mean of 0.88± 0.05 µg .hr /ml. While the AUC (0-∞) after product B administration was in the range of 0.81 to 0.96 µg .hr /ml, with a mean of 0.90± 0.o.05 µg .hr /ml.

Table (5): Pharmacokinetic Parameters of Enalapril Following the Oral Administration of Product B.

Subject	*Cmax (µg /ml)*	*t ½ (hr)*	*K (hr-1)*	*tmax (hr)*	*AUC (0-t) µg.hr/ ml*	*AUC (0-∞) µg.hr/ ml*
1	0.310	4.94	0.14	2.0	1.51	2.22
2	0.330	4.73	0.15	2.0	1.52	2.22
3	0.350	10.04	0.07	2.0	1.57	2.24
4	0.310	2.21	0.31	2.0	1.55	2.30
5	0.330	2.33	0.30	2.0	1.55	2.22
6	0.350	2.5	0.28	2.0	1.53	2.18
7	0.330	2.9	0.24	2.0	1.52	2.21
8	0.319	3.33	0.21	2.0	1.51	2.23
9	0.317	2.41	0.29	2.0	1.53	2.28
10	0.320	6.73	0.10	2.0	1.53	2.20
11	0.350	6.58	0.11	2.0	1.57	2.27
12	0.330	4.81	0.14	2.0	1.50	2.22
13	0.320	4.94	0.14	2.0	1.50	2.19
14	0.319	4.81	0.14	2.0	1.50	2.14
15	0.310	7.96	0.09	2.0	1.52	2.23
16	0.330	5.33	0.13	2.0	1.51	2.21
17	0.350	5.06	0.14	2.0	1.53	2.20
18	0.320	4.81	0.14	2.0	1.51	2.21
19	0.340	6.11	0.11	2.0	1.52	2,26
20	0.360	5.81	0.12	2.0	1.53	2.30
21	0.310	6.50	0.11	2.0	1.53	2.23
22	0.330	5.00	0.14	2.0	1.53	2.21
23	0.350	5.81	0.12	2.0	1.50	2.20
24	0.360	10.87	0.06	2.0	1.58	2.35
Mean	**0.331**	**5.27**	**0.16**	2.0	**1.53**	**2.23**
± S.D	**0.016**	**2.21**	**0.07**	**0.00**	**0.02**	**0.04**
Range	**0.31-0.360**	**10.87-2.21**	**0.31-0.06**	**2.0-2.0**	**1.50-1.58**	**2.14-2.35**
Media				**2.0**		

Cp max: Peak plasma concentration.
tmax : Time to reach peak plasma concentration.
AUC(0-t): Area under the plasma concentration time curve from time zero to 24 hours.
AUC(0-∞) : Area under the plasma concentration time curve from time zero to infinity.

The AUC (0-t) of hydrochlorothiazide following the administration of product A ranged from: 0.62 to 0.72 µg .hr /ml, with a mean value of 0.67± 0.03 µg .hr /ml. Whereas, AUC (0-t) of hydrochlorothiazide following the administration of product B ranged from: 0.065 to 0.74 µg .hr /ml, with a mean value of 0.69± 0.02 µg .hr /ml.

The t 1/2 of elimination of hydrochlorothiazide following the administration of product A Tablets ranged from: 2.64to 8.19 hours, with a mean value of 4.67± 1.60 hours. The t1/2 of elimination of hydrochlorothiazide following the administration of product B Tablets ranged from: 2.21to 6.97 hours, with a mean value of 4.11± 0.1.61 hours.

Table (6): Pharmacokinetic Parameters of Hydrochlorothiazide Following the Oral Administration of Product A.

Subject	*Cmax (µg /ml)*	*t ½ (hr)*	*K (hr-1)*	*Tmax (hr)*	*AUC (0-t) µg.hr/ ml*	*AUC (0-∞) µg.hr /ml*
1	0.056	4.5	0.15	3.0	0.68	0.91
2	0.058	6.9	0.10	3.0	0.72	0.96
3	0.059	3.8	0.18	3.0	0.69	0.91
4	0.056	4.9	0.14	3.0	0.65	0.84
5	0.059	3.5	0.19	3.0	0.69	0.90
6	0.057	2.6	0.26	3.0	0.68	0.90
7	0.058	8.1	0.08	3.0	0.66	0.88
8	0.056	5.5	0.13	3.0	0.69	0.91
9	0.059	4.3	0.16	3.0	0.66	0.87
10	0.057	3.0	0.23	3.0	0.68	0.9
11	0.056	4.3	0.16	3.0	0.71	0.95
12	0.059	6.7	0.10	3.0	0.65	0.86
13	0.058	4.1	0.17	3.0	0.72	0.96
14	0.056	2.9	0.24	3.0	0.68	0.9
15	0.056	3.8	0.18	3.0	0.68	0.9
16	0.056	6.9	0.10	3.0	0.65	0.85
17	0.056	3.5	0.20	3.0	0.62	0.79
18	0.054	6.9	0.10	3.0	0.68	0.91
19	0.056	3.8	0.18	3.0	0.65	0.86
20	0.058	3.6	0.19	3.0	0.62	0.80
21	0.054	4.1	0.17	3.0	0.65	0.84
22	0.056	3.6	0.19	3.0	0.62	0.80
23	0.054	3.0	0.23	3.0	0.65	0.85
24	0.056	6.9	0.1	3.0	0.63	0.82
Mean	**0.057**	**4.67**	**0.16**	**3.0**	**0.67**	**0.88**
± S.D	**0.002**	**1.6**	**0.05**	**0.00**	**0.03**	**0.05**
Range	**0.054-0.059**	**2.64 - 8.19**	**0.08-0.26**	**3.0-3.0**	**0.62-0.72**	**0.79-0.96**
Media				**3.0**		

Cp max: Peak plasma concentration.
tmax : Time to reach peak plasma concentration.
AUC(0-t): Area under the plasma concentration time curve from time zero to 24 hours.
AUC(0-∞) : Area under the plasma concentration time curve from time zero to infinity.

Table (7): Pharmacokinetic Parameters of Hydrochlorothiazide Following the Oral Administration of Product B.

Subject	*Cmax (µg /ml)*	*t ½ (hr)*	*K (hr-1)*	*Tmax (hr)*	*AUC (0-t) µg.hr/ml*	*AUC (0-∞) µg.hr/ml*
1	0.05	4.5	0.15	3.0	0.65	0.86
2	0.05	6.9	0.10	3.0	0.72	0.97
3	0.05	3.8	0.18	3.0	0.72	0.95
4	0.05	2.2	0.31	3.0	0.66	0.88
5	0.05	2.3	0.3	3.0	0.71	0.93
6	0.05	2.5	0.28	3.0	0.70	0.92
7	0.05	2.9	0.24	3.0	0.74	0.96
8	0.05	3.3	0.21	3.0	0.69	0.90
9	0.05	2.4	0.29	3.0	0.71	0.91
10	0.05	3.0	0.23	3.0	0.69	0.92
11	0.05	4.3	0.16	3.0	0.69	0.91
12	0.05	6.7	0.10	3.0	0.70	0.93
13	0.05	4.1	0.17	3.0	0.68	0.90
14	0.05	2.9	0.24	3.0	0.73	0.96
15	0.05	3.8	0.18	3.0	0.65	0.85
16	0.05	6.9	0.10	3.0	0.68	0.86
17	0.05	3.5	0.20	3.0	0.70	0.92
18	0.05	6.9	0.10	3.0	0.66	0.86
19	0.05	3.8	0.18	3.0	0.68	0.89
20	0.05	3.6	0.19	3.0	0.72	0.94
21	0.05	4.1	0.17	3.0	0.67	0.82
22	0.05	3.6	0.19	3.0	0.72	0.83
23	0.05	3.0	0.23	3.0	0.68	0.84
24	0.05	6.9	0.10	3.0	0.69	0.81
Mean	**0.057**	**4.11**	**0.19**	3.0	**0.69**	**0.90**
± S.D	**0.002**	**1.61**	**0.06**	**0.00**	**0.02**	**0.05**
Range	**0.054 - 0.059**	**2.21 - 6.97**	**0.10-0.31**	**3.0-3.0**	**0.65-0.74**	**0.81-0.97**
Media				**3.0**		

Cp max: Peak plasma concentration.
tmax : Time to reach peak plasma concentration.
AUC(0-t): Area under the plasma concentration time curve from time zero to 24 hours.
AUC(0-∞) : Area under the plasma concentration time curve from time zero to infinity.

The elimination rate constant K of hydrochlorothiazide following the administration of product A Tablets ranged from: 0.08 to 0.26 hour-1, with a mean value of 0.16 ± 0.05 hour -1. The elimination rate constant of hydrochlorothiazide following the administration of product B Tablets ranged from: 0.10 to 0.31hour-1, with a mean value of 0.19 ± 0.06 hour –1.

The percentage relative bioavailability of hydrochlorothiazide from product B Tablets compared to product A (standard) was found to be 102.14 % as determined by the ratios between the AUC (0-∞) of product B to product A.

Statistical analysis for the different pharmacokinetic parameters AUC (0-∞), Cmax, tmax , t ½ and K was performed. Statistical analysis was performed according to one way ANOVA using EXCEL software to determine the F value between the tested parameters. The results show that there is no significant difference between product A and product B, where the calculated f values were found to be less than the tabulated f values for all the tested pharmacokinetic parameters, namely AUC (0-∞), C max , tmax , t ½ and elimination rat constant (K) , indicating that both product are bioequivalent.

CONCLUSION

In conclusion, the HPLC method used is simple, sensitive , reliable and specific and could be used for pharmacokinetic and bioavailability studies of combination product of enalapril maleate and Hydrochlorothiazide. The bioequivalence study showed that product A and B are bioequivalent, since they deliver equivalent amounts of enalapril maleate and Hydrochlorothiazide to the systemic circulation at the same rate. The statistical analyses are AUC (0-∞), Cmax, tmax , t ½ and elimination rat constant (K) also showed that the two products are bioequivalent.

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