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Research Article

THERAPEUTIC EFFICACY OF HYDROCHLOROTHIAZIDE WITH LOSARTAN AS A FIXED DOSE COMBINATION BY LC-MS/MS

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ABSTARCT

The main aim of the present research work is to evaluate the pharmacokinetic parameters as well as therapeutic efficacy of Hydrochlorothiazide along with Losartan used as a fixed dose combined drug in treatment of hypertension. This study is carried out by utilizing a typical two periods; randomized and two way complete cross over design. All the 6 volunteers are received fixed dose and single dose product based on the randomization code in each clinical period. Then blood samples are taken and the comparative Pharmacokinetic study was carried out. Matrix effect for internal standard (Letrozole and Candesartan) and analytes (Losartan, LCA & HTCZ) were also carried out as per US-FDA guideline. Maximum plasma concentration of fixed dose combining drug (in which Losartan combined with HCTZ) was found 49 ± 8.87 ng/ml (C_{max}) at time 2.75 ± 0.25 hours (t_{max}) for HCTZ and the average maximum plasma concentration of 6 volunteers was 49.87 ng/ml and T_{max} 2.75 hours, AUC_{0-t} was 227.02 ng/ml at 48 hours and $AUC_{0-\alpha}$227.67 ng/ml at infinite time and plasma half-life ($T_{1/2}$) of HCTZ 4.91 hours and K_{et} value is 0.14 $hour^{-1}$. The matrix effect of internal standard (letrozole) ranged between 87.32% - 89.71% and for HCTZ it was between 88.47% - 89.86%. And the matrix effect of internal standard (Candesartan) ranged between 93.77% - 98.28% and same was found between 95.97% - 96.78% in case of Losartan and for LCA it was 95.41% - 98.39%. From the comparative pharmacokinetic study it can be concluded that after fixed dose combined drug treatment the maximum plasma concentration of losartan and losartan carboxylic acid were too much higher than the maximum concentration of losartan and LCA after single drug containing losartan only. And fixed dose combination drug is more susceptible than single dose of Losartan for the treatment of hypertension with better therapeutic efficacy.

Keywords: Fixed Dose, Pharmacokinetic study, LCA, Hypertension, C_{max}, $t_{max,}$ $T_{1/2}$

INTRODUCTION

The main rationale of this study is to estimate the pharmacokinetic parameters of FDC containing Losartan (50 mg each) and Hydrochlorothiazide (12.5 mg) where metabolite Losartan Carboxylic Acid (LCA) is formed in plasma due to presence of HCTZ and to evaluate the therapeutic efficacy in purview of pharmacological action. This LCA (the active metabolite) plays an important role compared to Losartan as a single dose towards therapeutic efficacy for hypertensive patients in 6 healthy human volunteers. This study is carried out by utilizing a typical two periods; randomized and two-way complete cross over design.

Hypertension is a long-term medical condition in which blood pressure in the arteries is persistently elevated[1]. This disorder leads to an increase in morbidity and mortality of patients not adequately controlled[2]. Long term high blood pressure may be the major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral vascular disease, vision loss, chronic kidney disease and even dementia[3]. CHD is the cause of death in 30% of males and 22% of females in England and Wales[4]. Hypertension may occur secondary to other disease process such as family history can develop hypertension with individual who is suffering from diabetes, obesity or disability status all are more likely to have hypertension than without. Besides environmental factors such as stressful lifestyle, high dietary intake of sodium and smoking can also predispose an individual to hypertension[5]. Blood pressure is expressed by two measurement i.e., systolic and diastolic pressure. For most of the adult's normal blood pressure at rest is within the range of 110-140 mmHg systolic and 80-90 mmHg diastolic[6]. High blood pressure is present if the resting BP is persistently at or above 140/90 mmHg. Arterial blood pressure plays direct role in cardiac output and peripheral vascular resistance[7]. These two are controlled mainly by two overlapping control mechanism: Baro-reflexes and Renin-Angiotensin-Aldosterone system (RAAS). Baro reflexes act by increasing sympathetic and decreasing parasympathetic output to heart resulting vasoconstriction and increased cardiac output. These changes result in the compensatory rise of blood pressure[8].

The renin is the aspartyl protease enzyme that cleaves the bond between the residue of 10 and 11 at amino acid terminus of angiotensinogen to generate Ang I[9]. The active form of renin is basically a glycoprotein (contains 340 amino acid) which is synthesized as a pre-pro enzyme (containing 406 amino acid residue) that is processed to pro-renin. This pro-renin and renin enhance the renin activity 4-5 times. They are stored along with renin in the juxtaglomerular cells and when released circulate in the blood. ARBs interrupt both the long and short loop negative feedback mechanism and increase the renin release with the help of angiotensinogen enzyme which is an abundant globular glycoprotein having molecular weight 55000-60000[10]. Angiotensinogen is synthesized and secreted from liver, certain region of CNS and kidney. This synthesis is stimulated by inflammation, insulin, estrogen, glucocorticoids, thyroid hormones and angiotensin II[11]. This angiotensin I is converted to angiotensin II by the help of ACE (angiotensin converting enzyme) which is an ectoenzyme and glycoprotein with an apparent molecular weight 170000. It is identical to kinase II, the enzyme that inactivates bradykinin and other vasodilator peptides. Angiotensin II act on AT1 receptor causing vascular growth (hyperplasia and hypertrophy), vasoconstriction and salt retention.

There are four main group of drugs are used for controlling hypertension they are Diuretics, sympathoplegic, vasodilators, drugs decreasing the action of RAAS and ARBs. In this study we explore the pharmacokinetic study of single dose (Losartan Potassium 50 mg) of drug side by side a fixed dose combination (containing Losartan Potassium 50 mg + Hydrochlorothiazide 12.5 mg) of drug on 6 healthy human volunteers suffering from hypertension. These drugs (both single and fixed dose) are administered to each volunteer in a defined process and their blood samples are taken and examined. LC-MS/MS technique is used to determine the concentration of Losartan, Losartan Carboxylic acid and HCTZ in human plasma after the administration of the drugs for fixed dose pharmacokinetic study.

MATERIAL AND METHODS

Chemicals and Reagents

Acetonitrile (MERK India Ltd., Mumbai), Methanol (MERK India Ltd., Mumbai), Isopropyl alcohol (MERK India Ltd., Mumbai), Water used in the entire analysis was prepared from Milli-Q water purification system produced from Millipore (Milli-Q A10, Bedford, MA, USA) until a resistively of 18.2 ΩM was achieved, The blank human plasma with EDTA-K3 collected from clinical pharmacological unit (CPU) of TAAB Bio-study Services, Kolkata and stored at -20ºC.

Instrumentation

Table 2: Instrumentation of devices used

Name of Instrument	Model
HPLC Pump	Shimadzu LC20AD
HPLC Auto-sampler	Shimadzu IL20AC
Triple Quadrupole Mass Spectrometer API 2000	AB Sciex Instrument
Centrifuge	REMI group
Evaporator	Home made
pH meter	Sartorious
Top loading balance	Sartorious

Drug Dosing

A two period, randomized two way complete crossover designs were undergone using the random number generator after the clinical and vital parameter examination. The marketed sample containing Losartan Potassium 50 mg + Hydrochlorothiazide 12.5 mg as a fixed dose drug and single dose drug containing Losartan Potassium 50 mg. Drugs were taken with 240 ml of drinking water on an empty stomach with at least 8-10 hours fasting condition in single dose without chewing. All the volunteers were received fixed dose and single dose product based on the randomization code in each clinical period as specified in Table 1. The patients are treated with two types of medicines- **A1:** Losartan (single dose) and **A2:** FDC (Losartan + Hydrochlorothiazide).

Table 1: Randomization of Dosing in Between 6 Volunteers

Phase 1			Phase 2		
Volunteers No.	**Volunteers Name**	**Treatment**	**Volunteers No.**	**Volunteers Name**	**Treatment**
1	V1	A1	1	V1	A2
2	V2	A1	2	V2	A2
3	V3	A2	3	V3	A1
4	V4	A2	4	V4	A1
5	V5	A1	5	V5	A2
6	V6	A2	6	V6	A1

Sampling Schedule and Blood Collection

Total 16 blood samples were taken as per the following schedule- 0,0.25,0.5,1,1.5,2,2.5,3,4,6,8,10,12,24,36 and 48 hours. The sampling was done by cubical vein puncture with installing of catheter. 5 ml of blood is taken at each time point.

Bio-analytical method development by gradation LC-MS/MS

Development of a LC-MS/MS method was essential to determine the concentration of Losartan and Losartan Carboxylic acid and Hydrochlorothiazide in human plasma after oral administration of the drugs for a fixed dose pharmacokinetic study. Chromatographic analysis was performed on a Shimadzu HPLC system equipped with LC-20AD Binary pump, SIL-20A Auto-sampler, CTO-10A Svp oven and CBM-20A Lite system Control Compartment. The developed method was validated as per

industrial guideline for the bio analytical method validation[12]. The chromatographic elution of analytes on Phenomenex Kinetex 5 µ C18 100A 50*3 mm column was initiated as a rapid, sensitive and rugged analytical method covering the dynamic linear range. The mobile phase (which is essential for synchronized determination of drug having pK_a values) methanol with ammonium acetate buffer having pH 6.5 shows higher response and enhancement of methanol content in mobile phase significantly increases the retention of losartan and losartan carboxylic acid, candesartan and thereby the analysis time. In order to achieve the better reproducibility and chromatography and superior noise ratio (>22) 10 Mm ammonium acetate buffer with 0.1%(v/v) ammonia solution together with Milli Q water having pH 8.9 at a flow rate of 0.5 ml/min is used. The chromatographic elution time for losartan, LCA, Candesartan (IS) was 2.4, 2.31, 2.27 min respectively in a run period of 7 min. This analysis was done by gradation method in which 0.01min to 1 min organic solvent 10% and then 1-4 min organic solvent 90% and then from 4min aqueous solvent 90% run up to 7 min for washing purpose.

For Hydrochlorothiazide the chromatographic elution is performed in same manner except here the acetonitrile- buffer mobile phase is used and the superior signal noise ratio and baseline resolution is obtained for analyte by replacing 10 Mm ammonium acetate buffer with 1 %(v/v) formic acid together with MilliQ water having pH 2.5 at a flow rate of 0.5 ml/min. The chromatographic elution time for HCTZ and Letrozole were 0.6, 0.97 respectively in a run time of 7 min. This analysis was done by gradation method in which 0.01-2.7 min organic solvent 40% and then 2.7-4.7 min organic solvent 60% and then from 4.7 min aqueous solvent 40% run up to 7 min for washing purpose.

Plasma Extraction and Sample Preparation

Plasma extraction was performed by protein precipitation technique.

A. Extraction Procedure of Losartan & Losartan Carboxylic acid

100 µl of plasma was taken and precipitated with 400 µl of MeCN containing 1000 ng/ml Candesartan (IS) and vortexed for 10 mins followed by centrifugation for 10 min at 12000 rpm at 4ºC. 300 µl supernatant was taken and transferred to auto-sample vials for injection.

B. Extraction Procedure of Hydrochlorothiazide

100 µl of plasma was taken and precipitated with 400 µl of MeCN containing 1000 ng/ml Letrozole (IS) and vortexed for 10 mins followed by centrifugation for 10 min at 10000 rpm at -20ºC. 300 µl supernatant was taken and transferred into auto-sampler vials for injection.

C. Stock solution and Calibration standards Preparation

Stock solution of Losartan, LSA, HCTZ and Candesartan (IS) and Letrozole respectively) were prepared by dissolving accurately weighed samples in DMSO to obtain concentration of 1 mg/ml. The stock solutions of Losartan and LCA were then gradually diluted with Methanol: Water in 50:50 (v/v) to obtain calibration samples.

D. Plasma Calibration Standards (ng/ml)

LCA: 50, 100, 200, 400, 800, 1600, 3200
Losartan: 9.37, 18.75, 37.5, 75, 150, 300, 600
HCTZ: 10, 20, 40, 80, 160, 320, 640

Validation of Method

The method validation was conducted in accordance with the guidelines US-FDA for selectivity, sensitivity, linearity, precision, accuracy, recovery and stability.

A. Specificity, selectivity and linearity

The specificity and selectivity was illustrated by the chromatograms of mobile phase run and extract of blank plasma recorded for the samples near the C_{max} for 2.00 to 3.00 hours for LCA and 1.00 to 2.50 hrs for Losartan and 2.5 to 3.00 hours for HCTZ. The linearity of calibration curve was determined by an un-weighted least square regression analysis. Representative calibration curves of Losartan, LCA and HCTZ from human plasma were depicted in linearity graph.

B. Precision and Accuracy

Between run precision and accuracy were determined from the low, medium and high QC samples. A total of 5 replicates of each QC concentration were assayed on day 1 and a total of each 5 replicates each QC concentration were assayed 0 day 2 and 3. The QC sample concentrations were determined from 3 different calibration curves that were assayed with QC samples LIN3 for Losartan, LIN2 for LCA and LIN1 for HCTZ. Precision was expressed as percent variation (%CV) where accuracy was measured as the percent nominal.

C. Stability

In the present study the freeze thaw, short term (ST), long term (LT) and auto-sampler (AS) stability had been performed as per the regulatory guidelines (US-FDA), and as per ICH guideline.

Comparative Pharmacokinetic study in Human volunteers

The comparative Pharmacokinetic study was carried out in 6 healthy Indian human volunteers of 32 ± 6 years of age (average) and 20.86 ± 1.48 kg/m^2 (average BMI) under fasting condition with single dose of and fixed dose administration. The volunteers were exposed in both the preparation i.e., single dose containing only Potassium and fixed dose containing Losartan Potassium with HCTZ. The clinical phase of this study was carried out in accordance with the supervisions of the Ethics Committee and all other pertinent requirement of ICH 'Guidance on Good Clinical Practice'.

RESULT AND DISCUSSION

Specificity, selectivity and linearity

From the plasma calibration standards (ng/ml) for Losartan, LCA, HCTZ, the proposed assay was found to be linear. The lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) were found 4.50 ng/ml, 9.37 ng/ml respectively for Losartan, 1.5 ng/ml and 50 ng/ml respectively for LCA and for HCTZ it were 1.00 ng/ml (LLOD) and 10 ng/ml (LLOQ).

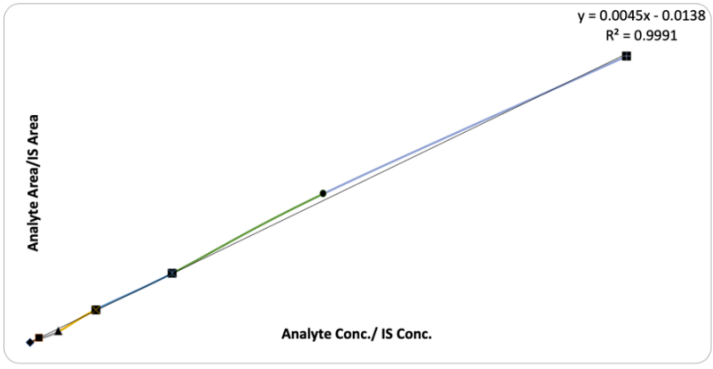

Figure 1: Calibration Curve of Losartan

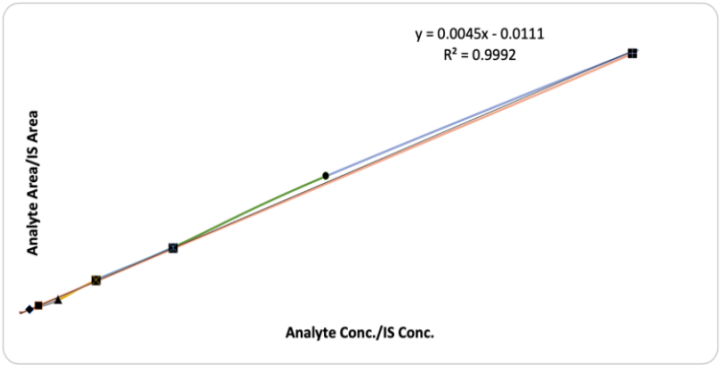

Figure 2: Calibration Curve of LCA

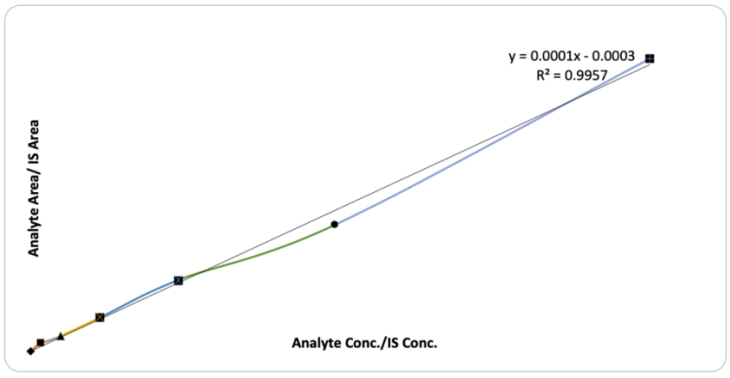

Figure 3: Calibration Curve of HCTZ

Precision and Accuracy

Between run precision values (%CV) ranged from 3.880% to 7.660% for Losartan, 4.592% to 6.583% for LCA and 5.123% to 7.839% for HCTZ. Between run accuracy values (% nominal) were 103.145% Losartan, 103.31% for LCA and 99.59% for HCTZ for low (LLOQ), 98.547% for Losartan, 103.14% for LCA and 99.25% for HCTZ for low QC (LQC), 94.174% for Losartan, 100.68% for LCA and 95.98% for HCTZ for medium QC (MQC) and 94.679% for Losartan, 100.99% for LCA and 102.22% for HCTZ for high QC (HQC) samples. The between run and within run precision results were represented in table below.

Table 3: Between run and within Run Accuracy and Precision of Losartan

	Between Run			Within Run		
	Mean ± SD	**% CV**	**Absolute bias**	**Mean ± SD**	**% CV**	**Absolute bias (%)**
LLOQ (9.37 ng/ml)	9.665 ± 0.74	7.66	103.145	10.088 ± 0.35	3.44	107.663
LQC (28.12 ng/ml)	27.711 ± 1.48	5.36	98.547	27.578 ± 1.68	6.10	98.073
MQC (225 ng/ml)	211.892 ± 8.22	3.88	94.174	217.294 ± 8.18	4.013	96.575
HQC (450 ng/ml)	426.054 ± 18.16	4.26	94.679	437.252 ± 21.34	4.88	97.167

Table 4: Between Run and Within Run Accuracy and Precision of LCA

	Between Run			Within Run		
	Mean ± SD	**% CV**	**Absolute bias (%)**	**Mean ± SD**	**% CV**	**Absolute bias (%)**
LLOQ (14.06 ng/ml)	13.819 ± 1.143	8.27	98.288	14.636 ± 1.33	9.08	104.097
LQC (42.18 ng/ml)	41.034 ± 1.189	2.89	97.283	41.360 ± 1.597	3.861	98.056
MQC (337.5 ng/ml)	321.164 ± 16.819	5.237	95.160	338.146 ± 11.525	3.408	100.191
HQC (675 ng/ml)	644.973 ± 20.581	3.191	95.552	662.918 ± 17.250	2.602	98.210

Table 5: Between run and within run Accuracy and Precision of HCTZ

	Between Run			Within Run		
	Mean ± SD	**% CV**	**Absolute bias (%)**	**Mean ± SD**	**% CV**	**Absolute bias (%)**
LLOQ (10 ng/ml)	9.959 ± 0.722	7.254	99.59	10.712 ± 0.336	3.134	107.12
LQC (30 ng/ml)	29.775 ± 2.334	7.839	99.25	29.946 ± 1.490	4.977	99.82
MQC (240 ng/ml)	230.351 ± 1.80	5.123	95.98	228.674 ± 16.35	7.150	95.28
HQC (480 ng/ml)	490.669 ± 30.557	6.228	102.22	460.492 ± 28.875	6.270	95.94

Stability

In bench top stability, QC samples were kept for 24 hours at room temperature and then processed and analyzed. Percentage stability was found within 103.14% to 109.17% for Losartan, 92.98% to 97.58% for LCA and 91.95% to 107.03% for HCTZ.

The auto sampler stability of Losartan ranged between 105.33% - 108.72%, LCA ranged between 94.92% - 97.80% and 90.31% - 104.33% for HCTZ.

In Freeze Thaw stability, the stability of low, medium and high quality control samples were determined after 3 cycles comparing against freshly thawed samples of the same concentration. The stability found for Losartan ranged between 105.20% - 107.72%, LCA ranged between 93.41% - 98.09% and 88.67% - 102.43% for HCTZ.

In Short Term stability, the percentage stability was found within 104.02% - 109.06% for Losartan, 92.18% - 98.19% for LCA and 93.17% - 103.00% for HCTZ.

In Long Term stability, the percentage stability ranged was found within 97.79% - 108.08% for Losartan, 91.16% - 92.86% for LCA and 95.00% - 102.46% for HCTZ.

Comparative Pharmacokinetic study in Human volunteers

After oral administration of Losartan Potassium 50 mg Tablet as single dose produced the maximum plasma concentration 217 ± 50 ng/ml (C_{max}) at time 1.75 ± 0.75 hours (t_{max}) for Losartan[13].

The average maximum plasma concentration of 6 volunteers is 207.93 ng/ml and T_{max} is 1.5 hours and AUC_{0-t} is 788.56 ng/ml at 48 hours and $AUC_{0-\alpha}$ is 791.34 ng/ml at infinite time. Plasma half-life of single dose Losartan was found to be 5.21 hour and elimination constant (K_{el}) was found 0.13 $hour^{-1}$. After oral administration of Losartan tablet 50 mg only approx. 14% losartan converted to its active metabolite Losartan Carboxylic Acid (LCA) in liver by hepatic enzyme and act on RAAS. The max plasma concentration produced in the body is 462.45 ± 62.22 ng/ml (C_{max}) at time 2.75 ± 0.25 hours (t_{max}) and the average maximum plasma concentration of 6 volunteers is 458.75 ng/ml and T_{max} 2.67 hours. And AUC_{0-t} is 2457.57 ng/ml at 48 hours. And AUC $_{0-\alpha}$ 2468.56 ng/ml at infinite time, plasma half-life $T_{1/2}$ of LCA 5.71 hours and elimination constant is 0.12 $hour^{-1}$.

On the other hand after administration of fixed dose combining drug in which Losartan combined with HCTZ (Losartan 50 mg + HCTZ 12.50 mg) it was found 49 ± 8.87 ng/ml (C_{max}) at time 2.75 ± .25 hours (t_{max}) for HCTZ and the average maximum plasma concentration of 6 volunteers was 49.87 ng/ml and T_{max} 2.75 hours, AUC_{0-t} was 227.02 ng/ml at 48 hours and $AUC_{0-\alpha}$227.67 ng/ml at infinite time and plasma half-life ($T_{1/2}$) of HCTZ 4.91 hours and K_{et} value is 0.14 $hour^{-1}$.

Table 6: Comparative studies of Pharmacokinetic Parameters

Drug/ Mean pK_a	**Losartan single dose**	**Losartan fixed dose**	**LCA single dose**	**LCA fixed dose**	**HCTZ fixed dose**
C_{max} (ng/ml)	207.93	383.59	458.74	543.56	49.87
T_{max} (hours)	1.50	1.25	2.67	2.42	2.75
AUC_{0-t} (ng.hr/ml)	788.56	1467.9	2457.57	1662.73	227.02
$AUC_{0-\alpha}$ (ng.hr/ml)	791.34	1473.02	2468.56	1666.18	227.67
K_{el} ($hour^{-1}$)	0.13	0.13	0.12	0.13	0.14
$T_{1/2}$ (hour)	5.21	5.53	5.71	5.35	4.91

Comparative Blood Pressure study in Human Volunteers

Before dosing each volunteers blood pressure was recorded called 0 hour dosing and then after dosing of drugs to each volunteers was recorded after 5 hour interval that is 5, 10, 15, 20, 30 hours. Measurement of blood pressure after single dose as well as fixed dose administration is shown in table below.

Table 7: Measurement of Blood Pressure after Single Dose Losartan administration

Time (hour)	Vol. 1 BP	Vol. 2 BP	Vol. 3 BP	Vol. 4 BP	Vol. 5 BP	Vol. 6 BP	Average BP
0	140/80	136/70	130/82	140/78	132/84	130/76	134/78
5	138/80	132/76	130/80	136/80	130/86	128/80	132/80
10	136/78	132/74	128/80	134/75	128/84	124/80	130/78
15	136/76	130/80	128/82	132/76	128/82	126/76	129/78
20	136/80	130/82	126/82	134/82	126/84	128/80	130/81
25	134/82	130/82	126/84	132/84	130/86	130/82	130/83
30	138/84	134/86	130/86	132/84	134/90	132/86	133/86

Table 8: Measurement of Blood Pressure after Fixed Dose Losartan with Hydrochlorothiazide administration

Time (hour)	Vol. 1 BP	Vol. 2 BP	Vol. 3 BP	Vol. 4 BP	Vol. 5 BP	Vol. 6 BP	Avg. BP
0	138/82	142/74	134/84	136/82	130/86	132/80	135/81
5	136/78	130/72	128/78	132/78	128/82	126/74	130/77
10	134/76	130/70	128/78	130/76	126/82	126/72	129/76
15	130/74	126/74	124/76	130/74	124/82	124/72	126/75
20	130/70	124/70	120/76	128/74	124/82	122/74	124/74
25	128/70	124/72	120/74	126/72	120/80	120/74	123/73
30	126/70	120/72	120/74	124/72	120/80	118/72	121/73

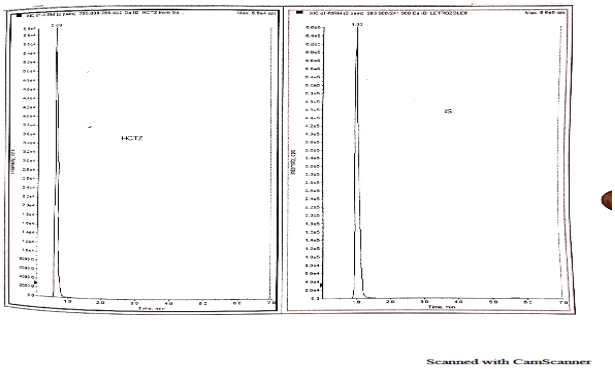

Figure 4: MRM (Multiple Reaction Monitoring) Chromatogram of Hydrochloride with Internal Standard Letrozole

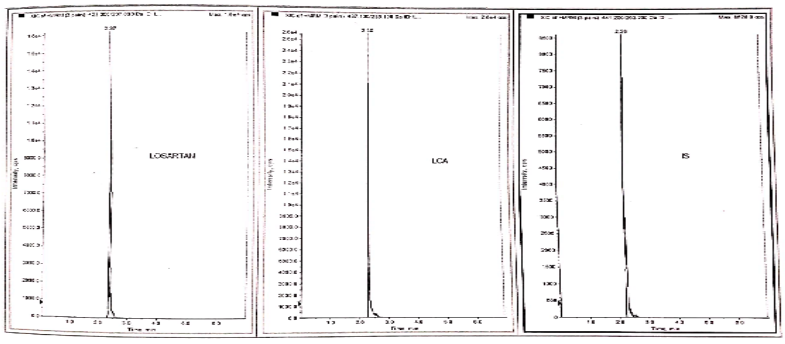

Figure 5: MRM (Multiple Reaction Monitoring) Chromatogram of Losartan, Losartan Carboxylic acid with Internal Standard Candesartan

CONCLUSION

From the comparative pharmacokinetic study it can be concluded that after fixed dose combined drug (Losartan with Hydrochlorothiazide) treatment the maximum plasma concentration of losartan and losartan carboxylic acid were too much higher than the maximum concentration of losartan and LCA after single drug containing losartan only. And fixed dose combination (Losartan with Hydrochlorothiazide) drug is more susceptible than single dose of Losartan for the treatment of hypertension. Besides Hydrochlorothiazide act as oxidizing agent with its degradation product and converts more efficiently from losartan to LCA in order to increase the action of AT1 blocker more than the single dose of losartan. LCA is 40 times more potent than losartan itself so during the treatment with fixed dose the systolic blood pressure is too much lower than with the treatment of single dose. Moreover, the side effect of Losartan and HCTZ as a single dose can be overcome to an extent by using fixed dose containing LCA and HCTZ and increase patient adherence. And there was no drug related adverse experiences were observed[14].

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Abbreviations

LC-MS/MS: Liquid Chromatography Quadruple Tandem Mass Spectrometry
AUC: Area under the Curve
API: Atomic Pressure Ionization
CHD: Chronic Heart Disease
RAS: Renin-Angiotensin system
ACE: Angiotensin Converting Enzyme
ARBs: Angiotensin receptor blocker
FDC: Fixed dose combination
$C_{max:}$ Maximum plasma concentration
T_{max}: Time to maximum concentration
$T_{1/2}$: Elimination half life
RO5: Rule of 5
HCTZ: Hydrochlorothiazide
LCA: Losartan Carboxylic acid
LLOD: Lower Limit of Detection
LLOQ: Lower Limit of Quantification
LQC: Low Quality Control
MQC: Medium Quality Control
HQC: High Quality Control
MRM: Multiple Reaction Monitoring

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