CATRINA (2009), 4 (1): 45 -52

Determination of Pioglitazone Hydrochloride in Human Plasma by High Performance Liquid Chromatography and Its Pharmacokinetics Application

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ABSTRACT

Development of a simple, rapid and routine assay of Pioglitazone Hydrochloride (PHCl) for the investigation of its pharmacokinetic parameters in human plasma and bioequivalence study of 45mg PHCl tablets manufactured locally (Test) and originally (Reference). After extraction of PHCl from plasma, it was chromatographed with mobile phase consisting of phosphate buffer: acetonitrile: methanol (65:25:10) at flow rate of 2ml/min and detected at wavelength of 235nm. The pharmacokinetic study was conducted in a 2 X 2 crossover design involving 24 volunteers. The criteria used to assess bioequivalence of the two products were $AUC_{(0-24)}$, $AUC_{(0-\infty)}$, C_{max}, and t_{max}. The described method for analysis showed that the recovery of PHCl from plasma was 99.84%, the limit of detection was 0.05µg/ml and the regression analysis for the drug concentrations indicated excellent linearity($r > 0.999$). Statistical analysis (ANOVA) of the measured parameters showed that there was no significant difference between the two products. The HPLC method presented is direct, simple, reproducible, sensitive and linear for the determination of PHCl in human plasma & is adequate for its clinical pharmacokinetic studies, besides, the Test was found to be bioequivalent to the Reference and both products can be considered interchangeable in medical practice.

Key words: Pioglitazone Hydrochloride, HPLC Determination, Analytical Assay, Human Plasma, Pharmacokinetics, Bioequivalence.

INTRODUCTION

Type 2 diabetes mellitus is a progressive and complex disorder that is difficult to treat effectively in the long term (Krentz and Bailey, 2005).Insulin resistance is a major pathophysiological mechanism in the development of type 2 diabetes and the traditional oral agents do not address the underlying insulin resistance responsible for the development of diabetes (Grossman, 2002).

The insulin-sensitizing thiazolidinedione class of antidiabetic agents has potentially advantageous effects on multiple components of the metabolic syndrome, preliminary data suggesting that thiazolidinediones (TZDs) may provide better long-term glycaemic stability (Krentz and Bailey, 2005). TZDs represent a relatively new class of oral hypoglycaemic medications that have been shown to reverse some of the metabolic processes believed responsible for the development of insulin resistance and, ultimately, type 2 diabetes. Pioglitazone is a TZD that reduces plasma glucose levels by increasing peripheral glucose utilization and decreasing hepatic glucose production (Grossman, 2002). Pioglitazone was approved by the FDA and commercialized in the U.S.A. in July 1999.The European Agency for the Evaluation of Medicinal Products gave its approval in fall 2000 with the same limitations of use as for rosiglitazone (Bailey, 2000).

TZDs when used as monotherapy or in combination therapy, not only reduce glycosylated hemoglobin levels, but also affect changes in blood lipid concentrations and have the potential to ameliorate cardiovascular disease risk (Olansky *et al.*, 2003).

An analytical method based on high-performance liquid chromatography (HPLC) with ultraviolet detection (269nm) was developed for the determination of pioglitazone in human plasma (Sripalakit *et al.*, 2006). Moreover, a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed and validated for the simultaneous determination of pioglitazone (PIO) and its two metabolites: M-III (keto-derivative) and M-IV (hydroxy-derivative) in human plasma; the method is simple, rapid and rugged, and has been applied successfully to sample analysis for clinical studies (Lin, 2003).

Hypoglycemic agents such as metformin, glipizide, glyburide, repaglinide, rosiglitazone, nateglinide, and pioglitazone are widely prescribed to control blood sugar levels. These drugs provide the basis for the development of a quantitative multianalyte bioanalytical method. As an example, a highly sensitive and selective multi-drug method based on liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed (Wang, 2007). Because they are metabolised via cytochrome P450 (CYP), glitazones are exposed to numerous pharmacokinetic interactions. However, in the absence of clinical data, it is prudent to reduce the dosage of each glitazone by half in patients treated with gemfibrozil. Conversely, pioglitazone do not seem to significantly affect the pharmacokinetics of other compounds. These compounds are generally used in combination with other pharmacological agents, thus, studies on pharmacokinetic interactions with the concurrent drug administered is required (Scheen, 2007).

The objective of this study is to develop a simple, sensitive, reproducible, and economic HPLC method with ultraviolet detection for the determination of PHCl in human plasma which would be validated for

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pharmacokinetics application as bioequivalence studies and also can be used for the investigation of pharmacokinetic interactions of PHCl in further clinical studies.

MATERIALS AND METHODS

Chemicals and Reagents

Standard PHCl powder was kindly supplied by October Pharma manufacture (Assay: 99.4%), Methanol and Acetonitrile of HPLC grade (Sigma, USA), Water of HPLC grade, Potassium dihydrogen phosphate (Riedel-de Haën, Germany), Orthophosphoric acid (Sigma, USA).

Equipment

The HPLC System consisted of:

1. An isocratic pump (Model LC-10AVD, Schimadzu, Japan).
2. Ultra-violet variable wavelength detector (Model SPD-10A, Schimadzu, Japan).
3. A rheodyne injector (Model 7161, Cotate California, USA equipped with 20 µL injector loop).
4. Degasser (Schimadzu degasser DGU-12A).
5. A C18 reversed-phase column, Phenomenenx Luna, 250 x 4.6 mm.
6. Guard column (Phenomenenx ODS, octadecyl 4 mm L x 3.0 mm ID),

- Centrifuge and Vortex mixer.

HPLC Assay

(a) Chromatographic conditions

Mobile phase composition is phosphate buffer: acetonitrile: methanol (65:25:10) V/V. pH 3 adjusted with 5% orthophosphoric acid. The flow rate was 2ml/min, the detection wavelength was 235nm. All assays were performed at ambient temperature.

(b) Stock and working standard solutions

The standard solution was prepared by dissolving 10mg of PHCl in 100 ml of methanol. The working standard solution was prepared by taking 10ml from the above solution in 100ml methanol (10 µg/ml).

(c) Calibration curve

Standard samples were prepared by transferring aliquots of the working standard solution at concentrations of PHCl ranging from (0.05 to 6) µg/ml into centrifuge tubes provided with tight sealing polyethylene caps, containing 1ml plasma.

(d) Sample preparation

One milliliter of acetonitrile was added to each tube, vortexted for 2 minutes and centrifuged for 15 minutes at 4000 rpm, the upper layer was transferred to another tube, filtered through a 0.45 um Millipore filter, 20 µl of the supernatant were injected on the column for analysis.

(e) Quantitation

Calibration curve of the peak area of PHCl was done. One ml of the different plasma samples obtained from the volunteers were treated as mentioned before.

The unknown sample concentration was calculated from the following formula:

$$Q = ({}^{R}/_{A} \pm B) \times \text{dilution factor}$$

Where, **Q** is the PHCl concentration, **R** is the peak area, **A** is the slope of the calibration curve and B is the Y-intercept. (Ahmed, 2001; Ahmed, 2002).

Bioequivalence study

(a) Subjects

Twenty-four healthy male volunteers participated in this study, they were subjected to physical examination, 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, the characteristics of the volunteers are summarized in Table (1). Subjects did not receive any meals for four hours after ingesting the tablets under study, neither any beverage drink, nor coffee or tea. At 11:00 a.m., they received a standard meal, and at 4:00 p.m. another meal. The written informed consent documents for the intended study were reviewed, discussed and then signed by the participant and clinical manager before the beginning of screening procedure without any obligation on the volunteers to continue if they did not want to.

(b) Study design

The study is a single blind, randomized, single-dose, two-treatment, two-period, two-sequence, crossover bioequivalence study under fasting conditions with 7-day washout interval between the doses. The number and disposition of blood collections as well as the wash-out period were designed with respect to pharmacokinetic parameters of PHCl.

(c) Sample Collection

The total of 15 blood samples were collected according to the following sample collection schedule: 5 ml at: 0,15 min., 30 min., 45min.,1,1.5, 2, 2.5, 3, 4, 6, 9, 12,15 and 24 hrs after dosing. The collected samples were immediately centrifuged and the plasma was frozen and stored at -20^{o}C until the analysis. Plasma samples labeled by protocol no., subject initials, study phase, treatment and sample time were forwarded to the analysis laboratory.

(d) Analysis of plasma samples

A simple and sensitive method for the determination of PHCl in plasma was used. The mobile phase consisted of 0.1 M phosphate buffer pH=3 (adjusted by 5% orthophosphoric acid): acetonitrile:Methanol

Table (1): Demographic data of the 24 Volunteers.

Code	Age (year)	Height (cm)	Weight (Kg)	Blood pressure (mmHg)	Pulse (Heart beat/min.)
A	23	174	80	100/70	90
B	21	170	75	120/85	75
C	22	185	87	120/90	85
D	23	181	85	110/80	84
E	22	183	90	120/90	75
F	23	176	69	110/80	72
G	25	168	73	100/70	83
H	23	190	92	120/85	84
I	21	167	70	120/90	76
J	22	180	88	110/80	70
K	22	175	69	120/85	98
L	22	188	90	110/75	88
M	22	180	74	120/90	86
N	22	169	72	110/80	65
O	21	168	79	120/90	90
P	23	180	70	110/90	85
Q	22	183	85	110/80	72
R	23	182	83	110/70	86
S	22	185	76	100/60	90
T	23	181	85	120/85	86
U	21	174	75	110/75	89
V	25	170	70	110/90	90
W	21	170	69	110/80	68
X	23	169	73	120/90	88

(65:25:10), and filtered through a 45 μm membrane filter. Separations were carried out at ambient temperature at a flow rate of 2 ml/min and detected by a UV detector at 235 nm.

(e) Pharmacokinetic Calculation

The following pharmacokinetic parameters of PHCl were assessed; maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), half-life of drug absorption ($t_{1/2ab}$) and absorption rate constant (K_{ab}), half-life of drug elimination during the terminal phase ($t_{1/2el}$) and terminal rate of elimination (K_{el}), area under plasma concentration-time curve from zero to the last quantifiable concentration estimate (AUC_{0-24}), and area under plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$). The pharmacokinetic parameters were calculated from the plasma level data obtained and presented as mean±S.D.

The relative bioavailability (F_{rel}) of the tested formulations was calculated by:

$$F_{rel} = AUC_{0-\infty} \text{ (tested formula)} / AUC_{0-\infty} \text{ (reference formula)} \times 100$$

(f) Statistical Analysis of Data

Statistical evaluation of the determined pharmacokinetic data was performed using a statistical computerized program SPSS Version 12 for the determination of analysis of variance (ANOVA).

Computation of the 90% confidence intervals by the parametric test Schuirmann's two one-sided test being applied to pharmacokinetic main parameters: C_{max}, AUC_{0-24} and $AUC_{0-\infty}$. The current evaluation criteria are based on the two one-sided test approach(Schuirmann's test), also commonly referred to as confidence interval approach or average bioequivalence, which determines whether the average values for the pharmacokinetic parameters measured after the administration of the Test and Reference products are comparable.To establish bioequivalence the AUC_{0-24}, C_{max} and t_{max} of the Test should not be less than 0.80 (80%) or greater than 1.25 (125%) of the Reference based on log-transformed data (bioequivalence limit of 80-125%).

RESULTS

HPLC Assay

Analytical Procedure and Validation

(a) Chromatogram of pioglitazone hydrochloride

PHCl was well separated and its retention time was 6.1 minutes. For this compound, sharp and symmetrical peaks was obtained with good baseline resolution and minimum tailing, thus facilitating the accurate measurement of the peak area.

(b) Linearity and detectability

Table (2) shows the peak area of varying amounts of PHCl (0.05–6) μg/ml, it was highly linear (r^2>0.998), the results of three replicate analysis of PHCl at three different days over one week period was obtained. From the results, the average correlation coefficient was 0.999 and the coefficient of variation of the slopes of the three lines is < 2%. The results, thus confirmed excellent

Table (2): Analytical precision for the analysis of PHCl performed on three sets standard curves of the same day.

Spiked Concentration (μg/ml)	Peak area			Mean	SD	RSD%
	1	2	3			
0.05	1479	1399	1398	1425.333	46.47939	1754.764
0.1	2955	2858	2835	2882.667	63.68935	3419.511
0.5	14123	14376	13921	14140	227.9759	27109.01
0.75	21241	21247	21112	21200	76.26926	11104.97
1.5	43484	42620	42142	42748.67	680.1892	140634.2
3	86976	83021	84115	84704	2042.228	594369.2
6	175805	169138	168452	171131.7	4061.734	1680261
R^2	0.9999	0.9999	0.9999	0.9999	1.36E-16	1.36E-16
Slope	29306	28098	28072	28492	705.0645	119011.9

linearity of the calibration plots and high reproducibility of the assay.

(c) Precision and accuracy

The precision, defined as the coefficient of variation of replicate analysis, and the accuracy, defined as the deviation between added and found concentration, of the assay of PHCl were detected. Table (3) represents the results of within day (intra-day) accuracy of the HPLC assay for the determination of PHCl in plasma, with an average recovery of 99.84% with a coefficient of variation of 1.97.

The results of the assay's accuracy for day to day (inter-day) showed an average recovery of 101.58% with an average coefficient of variation of Average CV% = 0.966 (Table 4). Day to day reproducibility data for the standard plots of PHCl in plasma are represented in Table (5) where the value of average correlation coefficient of three calibration plots ranged from 0.9998 to 0.9999.

The results of freeze–thaw stability of PHCl in plasma, short–term stability, long–term stability of PHCl in plasma and long–term stability of PHCl in stock solution are represented Tables (6 to 9).

Bioequivalence Study

(a) Clinical Observation

All the participating volunteers well tolerated the drug and the procedure adopted in the study. Every sample from the 24 volunteers during each phase was obtained at the proper time. No serious adverse event, or unexpected adverse drug reactions occurred during the study. Two AE's (fatigue) were observed in period (I), and one AE was observed in period (II) noted as fatigue.

(b) Plasma concentration–time data

The pharmacokinetic parameters determined for both products are summarized in Tables (10 and 11). The mean plasma concentration time curves of PHCl for the Reference and Test are shown in Figures (1 and 2) respectively.

(c) Assessment of bioequivalence

The assessment of bioequivalence, as a measure of efficacy, was based on the pharmacokinetic parameters derived individually for each participant from the PHCl concentration in plasma. The peak plasma concentration (C_{max}) of PHCl following the administration of Reference ranged from 1.54 to 1.87µg/ml (mean of 1.715±0.092µg/ml), whereas, the peak plasma concentration of PHCl following the administration of Test ranged from 1.51 to 1.82 µg/ml (mean of 1.703±0.081 µg/ml).The time to peak concentration (t_{max}) were 1.708 hrs and 1.770 hrs for Reference and Test respectively, while the mean $AUC_{0-\infty}$ for Reference and Test were 15.055±4.073µg.hr/ml and 14.165±3.437µg.hr/ml respectively. The percentage relative bioavailability of PHCl from Test compared to Reference was found to be 94.086% as determined by the ratios between the $AUC_{(0-\infty)}$ of Test to Reference.

Table (3): Within day accuracy for the determination of PHCl in Plasma.

PHCl (µg/ml)	n[a]	Mean recovery (µg/ml)	SD	Recovery %	CV %
0.05	3	0.050026	0.001631	100.0515	3.260949
0.1	3	0.101175	0.002235	101.1746	2.20939
0.5	3	0.49628	0.008001	99.25593	1.612276
0.75	3	0.744069	0.002677	99.20913	0.359761
1.5	3	1.500374	0.023873	100.025	1.591135
3	3	2.972905	0.071677	99.09682	2.411018
6	3	6.006306	0.142557	100.1051	2.373455

a = Number of replicate samples, Average recovery % = 99.84%. Average CV % = 1.97

Table (4): Between days accuracy for the determination of PHCl in Plasma.

PHCl (µg/ml)	n[a]	Mean recovery (µg/ml)	SD	Recovery %	CV %
0.05	3	0.052283	0.000865	104.5656	1.653806
0.1	3	0.10124	0.001317	101.2396	1.30075
0.5	3	0.514126	0.009981	102.8252	1.941348
0.75	3	0.758117	0.001055	101.0822	0.139179
1.5	3	1.505437	0.014214	100.3625	0.944195
3	3	3.029966	0.021529	100.9989	0.710525
6	3	5.99825	0.004573	99.97084	0.076247

a = Number of replicate samples. Average recovery %=101.58%. Average CV % = 0.966.

Table (5): Day to day reproducibility data for the standard plots of PHCl in Plasma.

Standard plot [a]	Slope	Intercept [b]	Correlation Coefficient
1	56936	836.72	0.9999
2	98172	470.26	0.9999
3	41408	248.11	0.9998

a = Obtained in 3 different days. b = the mean of 3-6 determinations at each drug concentration.

Table (6): Freeze–Thaw stability of PHCl in Plasma.

Added concentration (µg/ml)	Measured conc. /Nominal conc. (%)			
	Replicate 1	Replicate 2	Replicate 3	Average
0.5	97.65	100.74	99.68	99.36
1.5	98.42	101.33	96.59	98.78
3	99.24	102.81	100.47	100.84

Table (7): Short–Term stability of PHCl in Plasma.

Added concentration (µg/ml)	Measured conc. /Nominal conc. (%)			Average
	Replicate 1	Replicate 2	Replicate 3	
0.5	98.52	96.81	99.95	98.43
1.5	100.74	97.45	99.01	99.07
3	101.36	97.22	98.51	99.03

Table (8): Long–term stability of PHCl in plasma.

Added concentration (µg/ml)	Measured conc./Added conc. % for days					
	0	1	2	3	8	15
0.5	98.32	95.26	97.48	101.22	97.68	96.85
1.5	95.33	98.56	100.88	99.84	101.20	99.14
3	97.68	95.25	94.11	99.58	98.80	98.43

Table (9): Long–term stability of PHCl in stock solution.

Day	0	1	3	5	10	15
Pioglitazone hydrochloride Recovery(%)	100	99.8	101.5	99.4	97.8	99.07

Table (10): Pharmacokinetics parameters of PHCl following oral administration of reference.

Subject	C_{max}	t_{max}	AUC_{0-24}	$AUC_{0-\infty}$	K_{ab}	$t_{1/2ab}$	K_{el}	$t_{1/2el}$
A	1.83	1.5	7.02	7.656	0.3248	2.13	0.079	8.757
B	1.75	1.5	6.44	7.171	0.265	2.611	0.083	8.347
C	1.87	2	8.36	9.44	0.244	2.838	0.074	9.353
D	1.63	2	11.16	12.41	0.290	2.383	0.088	7.8735
E	1.58	2	13.16	14.52	0.255	2.714	0.102	6.766
F	1.57	1.5	10.22	10.56	0.317	2.180	0.147	4.686
G	1.73	2	13.45	14.39	0.450	1.539	0.117	5.882
H	1.62	1.5	13.65	14.19	0.398	1.737	0.147	4.707
I	1.54	1.5	10.26	10.71	0.349	1.982	0.132	5.215
J	1.81	2	18.04	19.38	0.3085	2.245	0.119	5.805
K	1.71	1.5	11.95	12.56	0.27	2.52	0.131	5.278
L	1.76	2	14.82	15.93	0.244	2.831	0.116	5.946
M	1.59	1.5	13.38	14.90	0.319	2.169	0.099	6.991
N	1.68	1.5	13.97	16.11	0.281	2.457	0.083	8.45
O	1.72	2	12.77	13.96	0.238	2.904	0.101	6.847
P	1.76	1.5	15.98	18.18	0.376	1.839	0.090	7.635
Q	1.82	2	15.90	17.09	0.370	1.872	0.117	5.921
R	1.73	1.5	15.86	18.38	0.276	2.507	0.083	8.318
S	1.64	1.5	14.21	16.05	0.294	2.350	0.092	7.499
T	1.8	2	18.95	20.91	0.242	2.853	0.106	6.487
U	1.79	1.5	17.68	19.89	0.274	2.521	0.094	7.299
V	1.72	1.5	14.83	16.80	0.337	2.050	0.091	7.608
W	1.83	2	19.32	23.29	0.238	2.902	0.075	9.153
X	1.69	1.5	15.44	16.75	0.376	1.8418	0.114	6.052
MEAN	1.715	1.708	13.62	15.05	0.3063	2.333	0.103	6.945
S.D.	0.092	0.251	3.45	4.07	0.057	0.403	0.021	1.369

Table (11): Pharmacokinetics parameters of PHCl following oral administration of test.

Subject	C_{max}	t_{max}	AUC_{0-24}	$AUC_{0-\infty}$	K_{ab}	$t_{1/2ab}$	K_{el}	$t_{1/2el}$
A	1.81	2	6.74	7.348	0.271	2.550	0.082	8.39
B	1.72	1.5	6.84	7.415	0.178	3.889	0.0868	7.977
C	1.78	2	6.9	7.883	0.206	3.354	0.066	10.38
D	1.73	2	12.69	13.30	0.344	2.009	0.1320	5.247
E	1.65	1.5	14.05	15.08	0.385	1.796	0.116	5.939
F	1.56	1.5	10.06	10.80	0.279	2.477	0.107	6.462
G	1.69	2	12.32	13.20	0.238	2.90	0.113	6.083
H	1.53	2	13.16	15.02	0.331	2.087	0.085	8.074
I	1.51	2	10.91	11.86	0.298	2.318	0.105	6.570
J	1.76	1.5	15.98	17.73	0.204	3.386	0.102	6.765
K	1.68	1.5	10.52	11.35	0.180	3.843	0.107	6.428
L	1.71	2	14.86	16.57	0.284	2.433	0.093	7.401
M	1.7	2	15.05	16.81	0.403	1.7195	0.096	7.168
N	1.67	1.5	12.74	13.90	0.239	2.891	0.103	6.715
O	1.82	2	13.12	14.48	0.208	3.323	0.095	7.257
P	1.72	2	13.71	15.48	0.254	2.727	0.090	7.642
Q	1.77	1.5	14.02	14.79	0.359	1.928	0.129	5.332
R	1.74	2	13.14	13.82	0.243	2.845	0.132	5.218
S	1.68	1.5	14.82	17.26	0.374	1.848	0.082	8.434
T	1.72	1.5	18.80	20.96	0.205	3.372	0.101	6.813
U	1.81	2	16.89	18.75	0.393	1.759	0.097	7.141
V	1.75	1.5	13.17	13.85	0.390	1.776	0.133	5.205
W	1.72	2	14.99	16.26	0.2100	3.299	0.110	6.254
X	1.65	1.5	15.36	15.97	0.271	2.556	0.149	4.631
MEAN	1.70	1.77	12.95	14.16	0.281	2.629	0.105	6.814
S.D.	0.08	0.25	3.05	3.437	0.074	0.685	0.019	1.298

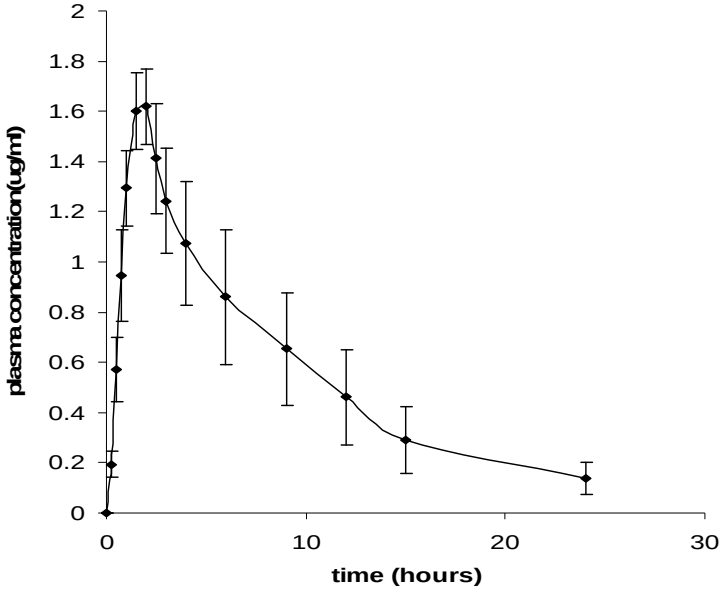

Figure (1): Mean plasma concentration time curve of PHCl following oral administration of reference product to 24 Volunteers.

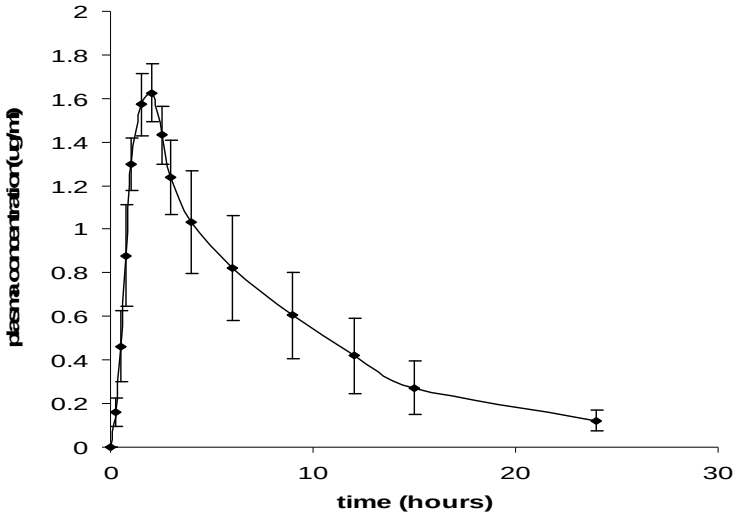

Figure (2): Mean plasma concentration time curve of PHCl following oral administration of test product to 24 Volunteers.

Moreover, the results (mean ± S.D.) of the Test/Reference (T/R) ratio applied to the pharmacokinetic main parameters, C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ were 0.993878±0.036209 µg/ml, 0.959975 ± 0.095907µg.hr/ml, and 0.953996 ± 0.113795µg.hr/ml.

(d) Statistical analysis

The data obtained from measurements of plasma concentration was transformed prior to analysis using natural logarithmic transformation. Pharmacokinetic parameters derived from these measurements, e.g.; $AUC_{0-\infty}$, C_{max}, t_{max}, K_{ab}, $t_{1/2ab}$, K_{el} and $t_{1/2el}$ were analyzed using ANOVA procedure using SPSS VERSION 12 COMPUTERIZED PROGRAM to rule out the possibility of a significant carryover effect. Applying ANOVA to test the significance difference between $C_{max,}$ $AUC_{(0-12)}$, $AUC_{0-\infty}$, t_{max}, $t_{1/2el}$, $t_{1/2ab}$, k_{ab}, k_{el}, C_{max}, $AUC_{(0-12)}$, $AUC_{0-\infty}$, t_{max}, $t_{1/2el}$,$t_{1/2ab}$,k_{ab},k_{el} for both products, the results showed that there is no significance difference between them (P-value = 0.633,1,0.417,10.735,0.075,0.202,0.814, 0.633 ,1, 0.417,1, 0.735, 0.075,0.202, 0.814) at $P < 0.05$. Also, the 90% confidence interval for AUC-ratio, C_{max}-ratio

and t_{max} ratio lied within an acceptance interval of 0.80-1.25 (bioequivalence limits set by the FDA).

DISCUSSION

The HPLC method used in this study was simple, of excellent sensitivity and specific, and could be used for pharmacokinetic and bioavailability studies of PHCl as the calibration curve was linear over the range 50-6000ηg/ml (r^2>0.998) similar to those results obtained by Sripalakit *et al.*, (2006) who find that the calibration curve was linear over the range of 50-2000ηg/ml (r^2>0.9987) and accordingly concluded that the method was of excellent sensitivity, accuracy, precision, recovery and stability, in addition, the assay has been applied successfully to a pharmacokinetic study with human volunteers.

Use of crossover designs for Bioequivalence studies allows each subject to serve as his or her own control to improve the precision of comparison. One of the assumptions underlying this principle is that *carryover effects* (also called *residual effects*) are either absent (the response to a formulation administered in a particular period of the design is unaffected by formulations administered in earlier periods) or equal for each formulation and preceding formulation.

The mean t_{max} for PHCl was found to be 1.7083±0.252 hours and 1.771±0.254 hours following the administration of Reference and Test respectively, in accordance with that obtained by Hanefeld M. (2001) who reported that Pioglitazone increases insulin sensitivity in target tissues, it is well-absorbed and reaching maximum concentrations in around 1.5 hours. Moreover, in another bioequivalence study, the median t_{max} for Pioglitazone test and reference tablets were found to be 1.50 h and 1.75 h. respectively (Wong *et al.*, 2004).

The results of the mean peak plasma concentration of PHCl (45mg) following the administration of Test and Reference (1.703±0.081µg/ml and 1.715±0.092µg/ml respectively), was higher than that obtained by Wong *et al.*, who found that the mean C_{max} of pioglitazone (30mg) ranged between 1.01microg/mL and 1.05microg/mL, which can be explained on the basis that the higher C_{max} value,obtained in this study, might be related to the higher dose of PHCl (45mg versus 30mg) ratio of 1.5:1. Similarly, the mean $AUC_{0-\infty}$ values for PHCl (45mg) were 14.165±3.437µg.hr/ml and 15.055±4.073µg.hr/ml for the test and reference tablets (45mg), respectively, in accordance with that obtained by Wong et al., where the mean AUC0-inf ranged between 10.89 microg x h/mL and 10.98 microg x h/mL for the test and reference tablets (30mg), respectively (Wong *et al.*, 2004).

In a bioequivalence study the ratios test/reference formulation for AUC_{0-inf}, AUC_{0-t} and C_{max} were 99.70%, 100.13% and 99.17%, respectively. Furthermore, the 90% geometric confidence intervals of the mean ratio of AUC0-inf were 90.59% to 109.72%, for AUC0-t, 90.69% to 110.55%, whereas for C_{max} they were 87.52% to 112.37% (Wong *et al.*, 2004). In this study the 90% geometric confidence intervals of the mean ratio of C_{max} were 98.19% to 100.59%, for AUC_{0-t} was 92.78% to 99.22%, and for $AUC_{0-\infty}$ was 91.58% to 99.22%, proving that the 90% confidence interval for these ratios lied within an acceptance interval of 0.80-1.25 (bioequivalence limits set by the FDA).

CONCLUSION

The HPLC method developed was simple, sensitive, accurate, precise and has been applied successfully to a pharmacokinetic and bioavailability studies with human volunteers. The bioequivalence study and the statistical analysis showed that Test and Reference products are bioequivalent, since they deliver equivalent amount of PHCl to the systemic circulation at the same rate. The percentage relative bioavailability of PHCl from Test compared to Reference was found to be 94.086%.

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Received July 30, 2008
Accepted January 25, 2009

تحديد البيوجليتازون هايدروكلورايد في بلازما دم الإنسان عن طريق الكروماتوجرافيا السائلة الفائقة الجودة وتطبيقها فى حركية الدواء

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الملخص العربـــى

إستهدفت هذه الدراسة تقييم ودراسة طريقة سريعة ودقيقة عن طريق الكروماتوجرافيا السائلة الفائقة الجودة لتحليل عقار البيوجليتازون هايدروكلورايد فى دم الإنسان وإستخدامها لمعرفة حركية الدواء والتكافؤ الحيوى للعقار مقارنة بالدواء المرجعى. فقد تم إستخدام محلول الفوسفات المنظم والأسيتونايترل والميثانول (بنسبة 65: 25: 10) كسائل متحرك- نازح، كما تم تثبيت الرقم الأيدروجينى عند 3 وتثبيت معدل مرور السائل النازح عند 2ملليتر/دقيقة، وطول الموجة المحددة عند 235 ن.م. وقد تم قياس كفاءة ودقة هذه الطريقة وقد أكدت التجارب حساسية ودقة وثبات النتائج إلي حد كبير عند تكراره في نفس اليوم أو فى أيام مختلفة.

دراسة حركية الدواء لهذا العقار تمت علي أربعة وعشرون متطوع بإستخدام 2 X 2 crossover design لتحديد بعض الثوابت الحركية للعقار مثل أعلى تركيز البيوجليتازون هايدروكلورايد بالبلازما (C_{max}) ، منحنى مستوي العقار فى الدم ($AUC_{(0-\infty)}$, $AUC_{(0-24)}$)، وقت ذروة التركيز (t_{max})، معدل إمتصاص العقار وفترة نصف العمر لإمتصاصه ($\mathbf{t_{1/2ab}}$,$\mathbf{k_{ab}}$) ومعدل التخلص من العقار وفترة نصف العمر للتخلص من البيوجليتازون هايدروكلورايد ($\mathbf{k_{el}}$,$\mathbf{t_{1/2el}}$). وقد أظهرت نتائج التكافؤ الحيوي للمنتج المحلي مقارنة بالمنتج الأصلى(منتج الشركة الأم) أن المستحضران متكافآن بنسبة 94.086%.