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DETERMINATION OF OXACEPROL (FED AND FASTING PERIOD) BY VALIDATED LC-MS/MS METHOD IN INDIAN HEALTHY HUMAN PLASMA AND ITS APPLICATION TO IN-VIVO COMPARATIVE PHARMACOKINETIC STUDY

Pallab Mandal[1], Tapan Kumar Pal[1]*, Soumya Chakraborty[1], Rakesh Bera[1], Chiranjit Saha[1] and Sanmoy Karmakar[2]

[1]TAAB Biostudy Services, Jadavpur, Kolkata 700032, India.

[2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

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***Corresponding Author**
Prof. Tapan Kumar Pal
TAAB Biostudy Services,
Jadavpur, Kolkata 700032,
India.

ABSTRACT

Aim and Objectives: The main aim of this study is to develop an LC-MS/MS method for quantifying a protein drug oxaceprol from human plasma. **Methods:** The human plasma and plasma extracted by protein precipitation technique. Oxaceprol was ionized at negative mode and m/z of oxaceprol was 171.9/130 and the internal standard was 269.0/170.0. The calibration concentrations were 62.5 to 8000ng/ml. This bio-analytical method which were successfully applied to comparative pharmacokinetic study and calculated Cmax, Tmax, T1/2, AUC 0-t, AUC 0-α, Kel, and secondary pharmacokinetic parameters like AUMC (ng.hr2./ml), MRT (hr.), Cl/F (ml/hr.), Vz/F (ml), Cmax/D(ng/ml/mg), AUC0-t/D (ng.hr./ml/mg), AUC0-infy/D(ng.hr./ml/mg) of test and reference preparation at fasting and fed condition. **Result:** After administration of the reference, preparation produced the maximum plasma concentration of 2905.51± 824.53 ng/ml (Cmax) at the time 2.21 ± 0.72 hr (Tmax) and 1169.39± 324.52 ng/ml (Cmax) at the time 2.19 ± 0.62 hr (Tmax) whereas after administration of the test preparation, as a single dose produced the maximum plasma concentration 2785.66 ± 734.73 ng /ml (Cmax) at the time 2.15 ± 0.65 hr (Tmax) and 1201.15 ± 340.83 ng /ml (Cmax) at the time 2.19 ± 0.62hr (Tmax) in fasting and fed state respectively. **Conclusion:** This is selective, sensitive, specific, reproducible, low ionization suppression, high recovery bio-analytical method which was successfully applied to a comparative pharmacokinetic study.

KEYWORDS: Oxaceprol, BA/BE study, Osteoarthritis, Anti-inflammatory drug, LC-MS/MS, Anti rheumatism.

INTRODUCTION

Oxaceprol is a derivative of L-protein and a DNA-encoded amino acid, under the class of a non-steroidal anti-inflammatory drug, used in the treatment of osteoarthritis.[8,9,11] Osteoarthritis is a most common disorder which affects the cartilaginous joints. To reduce the pain and for increasing mobility rehabilitation and physiotherapy were always prescribed for overcoming the degeneration of joints and inhibiting muscle atrophy.[10] The main pharmacological action of oxaceprol is that it inhibits the inflammation of the joints, especially the knee and hip joints by reducing the leukocyte accumulation in the joints but not inhibiting the synthesis of prostaglandins.[1,12] The CAS number of oxaceprol is 33996-33-7 and chemical name N-Acetyl-L-hydroxyproline and chemical formula C7H11NO4, molecular weight 173.17 (monoisotopic mass 173.06) In previous all research workers published articles of oxaceprol depending on its efficacy and pharmacokinetic study on rat plasma but the main aim of this study is to developed a simple, sensitive, specific and reproducible validated bioanalytical LC-MS/MS method as per US-FDA and EMA guidelines for determination of oxaceprol from human plasma.[2,3] Previously nimesulide was used as an anti-inflammatory drug which was a specific COX-2 inhibitor but now this drug is totally banned for hepatotoxicity and several other adverse drug reaction.[5] From the previous literature review, it was observed that any papers in any journals regarding pharmacokinetic parameters of oxaceprol were published. This is the first study till now about the detailed pharmacokinetic study of oxaceprol in Indian human plasma by liquid chromatography-mass spectrometry.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

The chemicals and reagents used for this analysis are acetonitrile and methanol (HPLC grade) and ammonia solution, formic acid (AR grade), and Milli Q water from the Milli Q water purification system.

2.2. Calibration standard concentration and quality control samples preparation

The prepared stock solution concentration of oxaceprol was 1mg/ml in DMSO solution. The internal standard was tolbutamide which stock solution concentration was 1mg/ml in DMSO solution. From this stock solution intermediate and working, the standard concentrated

solution was prepared by dilution with methanol: acetonitrile mixed with 2:1 with Milli Q water. Then the plasma calibration concentration was prepared by adding 500µl working standard mixed with 500µl human plasma. The calibration concentrations were 62.5, 125, 250, 500, 1000, 2000, 4000, 8000 ng/ml and LQC 187.5ng/ml, MQC 3000ng/ml, HQC 6000ng/ml and LLOQ was 62.5ng/ml.

2.3. Liquid chromatography quadrupole tandem mass spectrometry (lc-ms/ms api-4000, qtrap) analysis

For quantification of oxaceprol from human plasma use Liquid Chromatography Quadruple Tandem Mass Spectrometry, Atmospheric Pressure Ionization (ESI) -4000, QTRAP of AB Sciex Instrument. For quantification Phenomenex Kinetex 5µ C18 100A 50*3mm LC-MS/MS column was used. The mobile phase used for analysis was 0.1% Ammonia Solution in Milli Q water with 5 mM Ammonium acetate as an aqueous solvent and 0.1% Formic acid in methanol: acetonitrile (2:1) as an organic solvent. For quantification, the gradient method was used in which 0.01min. to 2.00min. used 40% organic solvent and from 2.00min. to 4.00min. used 60% organic solvent and from 4.00min. to 7.00min. used again 40% organic solvent as washing purpose at 0.5ml/min flow rate. The retention time of oxaceprol was 0.65min. and 1.85min. for IS.

2.4. Plasma sample preparation

Plasma extraction was performed by the Protein precipitation technique, 100 µl of plasma was taken and precipitated with 400 µl of MeCN containing 500 ng/ml Tolbutamide (IS) and vortexes for 10 mins, followed by Centrifugation for 10 min. at 10,000 rpm at -20°C. 300µl. The supernatant was taken and diluted 10 times, then transferred into autosampler vials for injection.

2.5. Method validation

The LC-MS/MS bioanalytical method of quantification of oxaceprol validated by US-FDA and EMA guidelines. The calibration concentrations range was 62.5ng/ml to 8000ng/ml and linearity-1,2 and 3 was done by eight calibration standard concentrations and 187.5ng/ml as LQC, 3000ng/ml, and 6000ng/ml as MQC and HQC respectively. In this set of bias 62.5ng/ml uses as an LLOQ sample. In LQC, MQC, and HQC six different samples of the same concentration were used in the same bias and reported as five samples. On each day freshly sample was prepared for linearity1,2 and 3. The sample stability was tested as freeze-thaw, short-term, autosampler, and benchtop stability. All samples were compared with

freshly prepared samples of the same day of analysis. The recovery and matrix effect of internal standard and oxaceprol was analyzed and calculated on basis of the area of analyte and IS. Long-term stability was done after 25days of freezing the plasma concentrated samples at -20ºc and compared with freshly prepared plasma concentrated samples.[4,6,7]

2.6. Ethical Guideline

This study was carried out in accordance with clinical research guidelines for medical research involving human subjects [59th WMA General Assembly, Seoul, October 2008] and as per ICMR and Indian GCP guidelines.

This oxaceprol study protocol and informed consent form, case record form, subject information sheet was submitted to the HURIP Independent Bio-ethics committee, Kolkata, India. CDSCO registration: ECR/103/Indt/WB/2013/RR-19 which is valid up to 21-Nov.-2024.

2.7. Pharmacokinetic study

This was a single-dose, randomized **[Table-1(a, b)]** two-treatment, and two-way cross-over study, with at least a washout period of 7 days between the two dosing sessions.

Table-1a: Randomization schedule under fasting condition.

Subject No.	PERIOD I	PERIOD II
1	A1	A2
2	A2	A1
3	A2	A1
4	A1	A2
5	A1	A2
6	A2	A1
7	A1	A2
8	A2	A1
9	A2	A1
10	A1	A2
11	A1	A2
12	A2	A1
13	A1	A2
14	A2	A1
15	A2	A1
16	A1	A2
17	A1	A2
18	A2	A1
19	A1	A2
20	A2	A1

21	A2	A1
22	A1	A2
23	A1	A2
24	A2	A1

A1 – Reference Preparation and A2 – Test Preparation

Table-1b: Randomization schedule under fed condition.

Subject No.	PERIOD I	PERIOD II
1	A1	A2
2	A1	A2
3	A2	A1
4	A1	A2
5	A2	A1
6	A2	A1
7	A1	A2
8	A1	A2
9	A2	A1
10	A1	A2
11	A2	A1
12	A2	A1
13	A1	A2
14	A1	A2
15	A2	A1
16	A1	A2
17	A2	A1
18	A2	A1
19	A1	A2
20	A1	A2
21	A2	A1
22	A1	A2
23	A2	A1
24	A2	A1

A1 – Reference Preparation and A2 – Test Preparation

In each dosing session, volunteers received either of the test preparation of Oxaceprol 600mg SR tablet (each film-coated sustained release tablet contains Oxaceprol 600mg) or the Reference preparation of Lupoxa® Capsules (each capsule contains Oxaceprol 600mg SR), only on the study day (as fasting and fed condition) at a fixed time. A total of 20 blood samples of each volunteer were collected at 0 hr. (before drug administration) and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 6.5, 7.0, 8.0, 10.0, 12.0, 12.5, 13.0, 14.0, 16 , 24, 48 and 72 hrs (after drug administration) in the EDTA containing test tubes with at each time point. The total number of participated volunteers was twenty-four and the BMI of each volunteer showed in **[Table-2].**

Table-2: BMI of each volunteer (n= 24), data given in mean±SD.

Vol. No.	Sex	Age	Height (cm)	Weight (kg)	BMI(kg/m^2)
1	M	29	163	55	20.70
2	M	26	154	55	23.19
3	M	38	154	52	21.93
4	M	30	163	60	22.58
5	M	30	169	67	23.46
6	M	26	160	52	20.31
7	M	22	158	51	20.43
8	M	25	168	58	20.55
9	M	33	164	65	24.17
10	M	43	159	52	20.57
11	M	25	158	50	20.03
12	M	35	156	55	22.60
13	M	34	169	65	22.76
14	M	22	161	54	20.83
15	M	26	157	51	20.69
16	M	34	171	59	20.18
17	M	43	160	55	21.48
18	M	28	164	55	20.45
19	M	42	166	56	20.32
20	M	40	155	57	23.73
21	M	24	166	56	20.32
22	M	32	157	60	24.34
23	M	37	166	66	23.95
24	M	20	157	60	24.34
Mean		**31.00**	**157.29**	**56.92**	**19.99**
S.D(±)		**6.73**	**6.77**	**5.05**	**1.62**

Volunteer blood samples were collected and after centrifuged separated plasma was stored frozen at –200C with appropriate labeling of volunteer code no with study date and collection time. Samples were analyzed by LCMS/MS (API 4000 QTRAP) after extracting the drug from plasma and injecting it on the LCMS/MS column for chromatographic analysis. Plasma levels of Oxaceprol for every volunteer at each time point were plotted to obtain Time-Plasma concentration curves for the study preparations. The mean pharmacokinetic parameters were calculated by using SAS 9.1.3 Version software.

3. RESULT

3.1. bio-analytical method development

Oxaceprol is an N-Acetyl-L-hydroxyproline and it is a carboxylic acid with and Pka value of 3.69. For better ionization of oxaceprol used 0.1% Ammonia Solution in Milli Q water with 5 mM Ammonium acetate as aqueous solvent and 0.1% Formic Acid in a mixture of methanol and acetonitrile in 2:1 as an organic solvent. For the quantitation of oxaceprol from

plasma and better sensitivity negative mode was selected. The parent ion (Q1) of oxaceprol was [M-H]- at m/z 171.9 and the product ion was m/z 130 [(M-CH3 CO-H) ¯] for releasing acetone**.[Figure-1]**

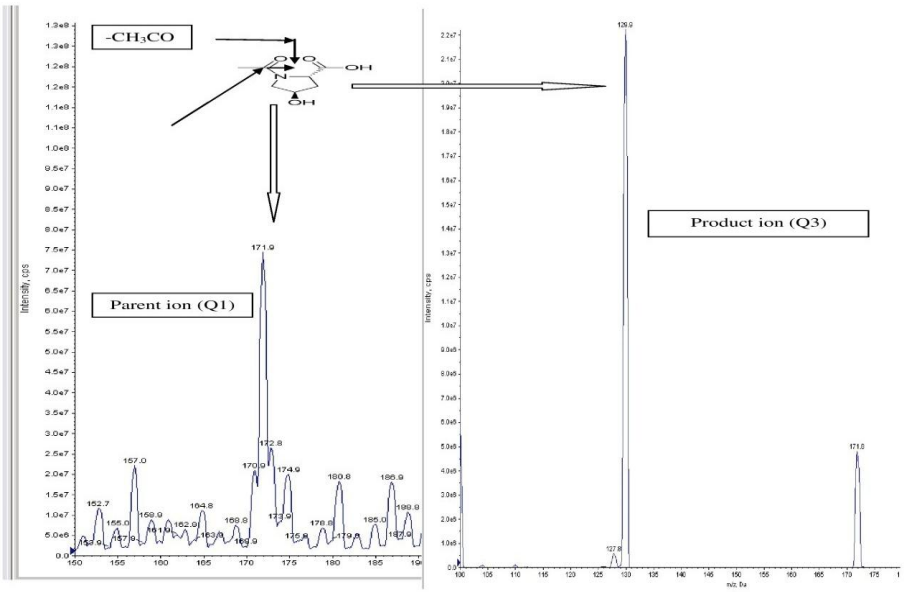


Figure-1: Parent ion (Q1) and product ion (Q3) scan of Oxaceprol

Figure-1: Parent ion (Q1) and product ion (Q3) scan of Oxaceprol.

The internal standard was tolbutamide. The parent ion (Q1) of tolbutamide was [M-H]- at m/z 269.0 and the product ion was m/z 170. For greater reproducibility and sensitivity the selected mobile phase was 0.1% Ammonia Solution in Milli Q water with 5 mM Ammonium acetate as aqueous solvent and 0.1% Formic Acid in methanol with acetonitrile in 2:1 ratio as organic solvent at a flow rate of 0.5000 ml/min. The optimized mass spectrometry parameters for oxaceprol and IS showed in **[Table-3].**

Table-3: Optimized mass spectrometry parameters for Oxaceprol and IS.

Parameter(s)	**Value**
Ionization mode	MRM (-ve)
Source temperature (ºC)	400
Dwell time per transition (msec)	200
Curtain gas (psi)	20
CAD gas (psi)	3
Ion spray voltage (V)	-4500.00
Ion source gas 1 (psi)	40
Ion source gas 2 (psi)	45
Focussing potential (V)	400
Declustering potential (V)	-41 (Oxaceprol) and -60 (IS)
Entrance potential (V)	-10

Collision energy (V)	-18 (Oxaceprol) and -25(IS)
Collision cell exit potential (V)	-15 (Oxaceprol and IS)
Transition pair of Oxaceprol (analyte)	172.0/130.0
Transition pair of Tolbutamide (IS)	269.0/170.0

For elution of oxaceprol, gradation method was performed in which 40 % organic solvent was used for 0.01 min to 2.00 min and 60% organic solvent used for 2.00 min to 4.00 min of total run time whereas 60 % aqueous solvent was used from 4.00 min to rest of the total run time (7.00min) for washing purpose. The gradient curve showed in **[Figure: 2].**

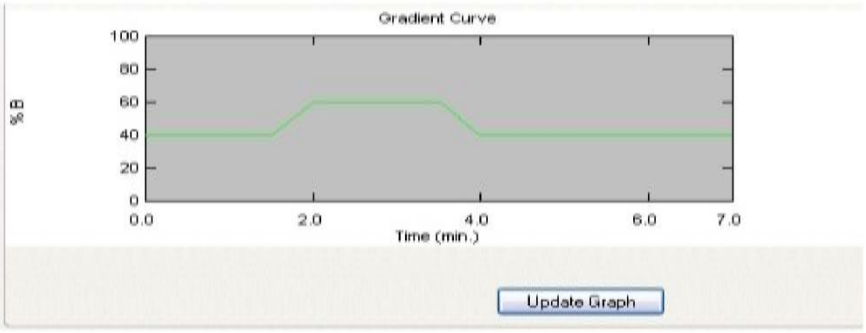


Figure-2: Gradient curve of method development of Oxaceprol

Figure-2: Gradient curve of method development of Oxaceprol.

Plasma was extracted by protein precipitation technique and for this reason recovery of this drug from plasma was 87.08% to 91.51% and in the case of internal standard, it was 90.39% to 99.25% **[Table-8(a, b)].**

Table-8a: Recovery of Oxaceprol.

INJ No.	Diluent Sample			In Plasma		
	LQC 187.5 ng/ml	MQC 3000 ng/ml	HQC 6000 ng/ml	LQC187.5 ng/ml	MQC 3000 ng/ml	HQC 6000 ng/ml
1	4750316.16	4598167.18	4212772.60	4505199.32	3881642.41	3847457.84
2	5308605.01	4685812.57	5112450.30	4872707.09	3990744.40	5495812.96
3	4695590.15	4461829.04	4276308.27	4238577.61	4094830.18	3571132.41
4	4410610.42	4627922.87	4569647.40	4007340.29	3971332.73	4173644.98
5	4591823.20	4559404.61	4250767.26	4009008.29	4031978.22	3429915.38
Mean	**4751388.99**	**4586627.25**	**4484389.17**	**4326566.52**	**3994105.59**	**4103592.71**
% Recovery				**91.06**	**87.08**	**91.51**

Table-8b: Recovery of IS.

INJ No.	Diluent Sample			In Plasma		
	LQC 187.5 ng/ml	MQC 3000 ng/ml	HQC 6000 ng/ml	LQC 187.5 ng/ml	MQC 3000 ng/ml	HQC 6000 ng/ml
1	9576481.81	9965772.62	7975233.40	8498481.38	8880068.39	8859991.94
2	9619123.92	9593272.96	7815211.23	8939775.07	8867580.65	8247471.52
3	9676111.26	9466372.22	8517740.08	8673734.93	8937093.49	8115143.48
4	9371911.94	9360864.06	8058814.21	8495924.93	8846871.55	8096364.77
5	9759629.56	9392947.71	8325793.74	8784523.90	9000156.92	7068261.56
Mean	**9600651.70**	**9555845.91**	**8138558.53**	**8678488.04**	**8906354.20**	**8077446.65**
% Recovery				**90.39**	**93.20**	**99.25**

3.2. Method validation

Oxaceprol was quantified from human plasma after administration of 600mg SR Tablet and the concentration of LLOQ was 62.5ng/ml where the LOD value was 1.0ng/ml. The nominal percentage of linearity was 94.26% to 108.65% **[Table-4a].**

Table-4a: Pre-study linearity of detector response (n=3), data given in mean±SD.

Linearity	Concentration (ng/ml)							
	62.5	125	250	500	1000	2000	4000	8000
LIN 1	63.31	124.53	254.51	447.96	948.42	1909.25	4363.46	8663.27
LIN 2	57.79	138.58	284.82	455.22	987.99	1851.83	4229.29	7566.18
LIN 3	63.79	112.35	275.58	510.72	1035.28	2048.77	3806.48	7564.97
Average	**61.630**	**125.153**	**271.637**	**471.300**	**990.563**	**1936.617**	**4133.077**	**793.473**
S.D	**3.334**	**13.126**	**15.535**	**34.331**	**43.487**	**43.487**	**43.487**	**43.487**
% C.V.	**2.664**	**10.488**	**5.719**	**7.284**	**4.390**	**2.246**	**1.052**	**0.548**
Nominal % NOMINAL	**98.61**	**100.12**	**108.65**	**94.26**	**99.06**	**96.83**	**103.33**	**99.14**

The mean regression value of three linearities was 0.9958 where the weighing factor was (1/X*X) and the linear regression formula was applied Y= m X + c **[Table-4b].**

Table-4b: Pre-study linearity of detector response statistics (n=3), data given in mean±SD

LINEARITY	STATISTICS		
LINEARITY CODE	SLOPE (m)	INTERCEPT (c)	R square
LIN 1	0.00021	-0.00110	0.9969
LIN 2	0.00023	-0.00012	0.9944
LIN 3	0.00027	0.00105	0.9972
MEAN	**0.00025**		**0.9958**
S.D.	**0.00003**		**0.00198**
C.V.%	**11.405**		**0.199**

And plasma calibration curve was shown in **[Figure: 3].**

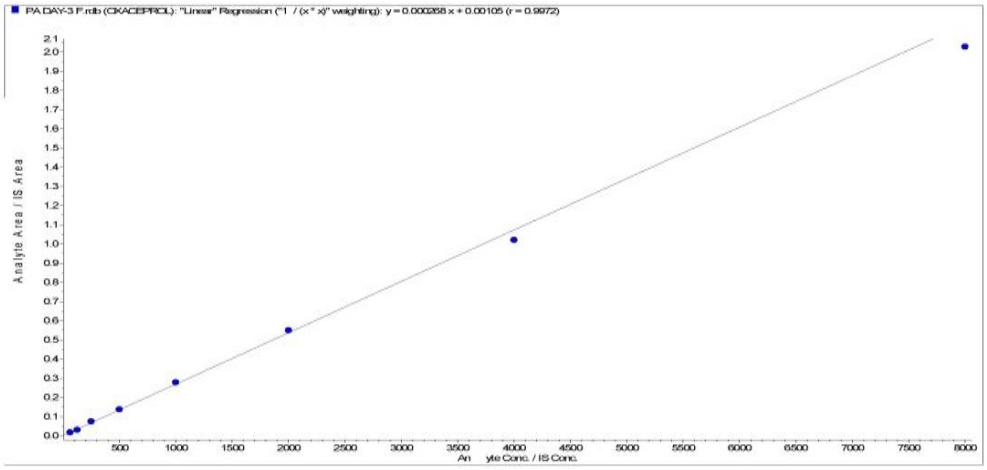


Figure-3: Plasma calibration curve of Oxaceprol

Figure-3: Plasma calibration curve of Oxaceprol.

The absolute percent bias between run precision and within run precision was 101.51% to 104.62% and 100.24% to102.91% respectively **[Table-5].**

Table-5: Precision and accuracy (n = 5), data given in mean±SD.

	Between run			Within run		
	Mean ± SD	**C.V.%**	**Absolute bias (%)**	**Mean ± SD**	**C.V.%**	**Absolute bias (%)**
LLOQ (62.5 ng/ml)	64.864±2.873	4.430	104.62	63.804±3.227	5.058	102.91
LQC (187.5 ng/ml)	190.339±7.024	3.690	101.51	187.946±5.703	3.034	100.24
MQC (3000ng/ml)	3089.667±137.393	4.447	102.99	3040.436±109.157	3.590	101.35
HQC (6000ng/ml)	6172.610±203.635	3.299	102.88	6081.164±211.594	3.480	101.35

The representative chromatograms of blank, blank with internal standard, LLOQ, LQC, MQC, HQC samples were shown in **[Figure: 4(a,b,c,d,e,f,g)].**

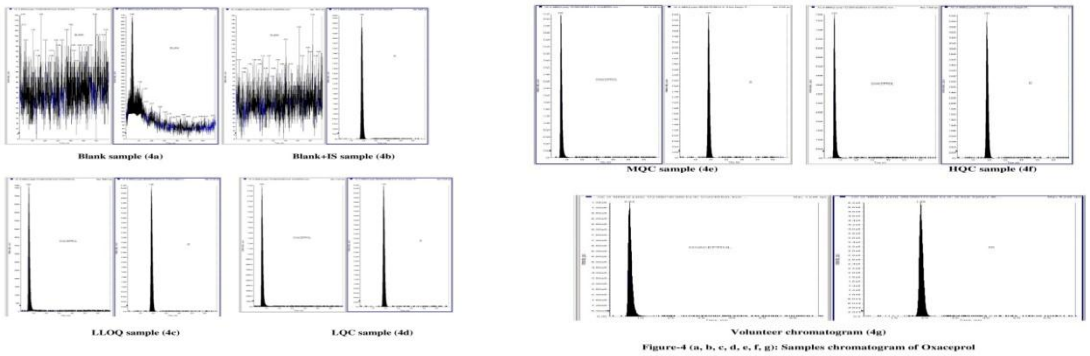


Figure 4: (a, b, c, d, e, f, g): Samples chromatogram of Oxaceprol.

The percentage of freeze-thaw stability after three freeze-thaw cycles, short term stability, benchtop, autosampler and long term stability was 92.78% to 100.26%; 91.07% to 98.40%; 97.16% to 98.90%; 95.78% to 99.62%; and 91.09% to 96.39% respectively **[Table-6].**

Table -6: Stability Study (Freeze thaw, Short term, Auto sampler, Bench top stability, Long term stability), n=5, data given in mean±SD.

		Inj No.	LQC (187.5 ng/ml)	MQC (3000 ng/ml)	HQC (6000 ng/ml)
	Freshly Thawed	1	194.65	3239.32	5852.12
		2	197.40	3226.03	6035.38
		3	199.44	3187.42	6097.85
		4	199.84	3216.57	6128.49
		5	194.63	3129.05	6300.23
		Mean	**197.19**	**3199.68**	**6082.81**
Freeze thaw stability	After 3 cycle	1	204.13	2877.59	5556.36
		2	196.42	3021.76	5778.04
		3	205.09	3019.15	5645.22
		4	179.25	2898.16	5886.31
		5	203.62	3027.18	5807.29
		Mean	**197.70**	**2968.77**	**5734.64**
	% Stability		**100.26**	**92.78**	**94.28**
Short term stability	After 24 hours.	1	188.82	2851.32	5772.32
		2	200.29	2854.9	5849.32
		3	193.79	2895.65	5789.53
		4	200.05	3009.21	5631.43
		5	187.25	2958.52	5739.68
		Mean	**194.04**	**2913.92**	**5756.46**
	% Stability		**98.40**	**91.07**	**94.63**
Auto sampler stability	After 24 hours in auto sampler (15^{o}c)	1	203.47	3058.36	6003.68
		2	202.19	3059.32	5956.71
		3	180.44	3046.25	6009.31

		4	201.79	3142.06	6005.15
		5	194.30	3017.99	6050.91
		Mean	**196.44**	**3064.80**	**6005.15**
	% Stability		**99.62**	**95.78**	**98.72**
Bench top stability	After 24 hours in laboratory room temperature	1	182.99	3098.64	6056.68
		2	195.49	3083.02	5974.50
		3	187.47	3233.71	6004.64
		4	191.61	3058.83	5940.28
		5	200.37	3132.69	6104.67
		Mean	**191.59**	**3121.38**	**6016.15**
	% Stability		**97.16**	**97.55**	**98.90**
Long term stability		1	190.51	2931.15	5478.5
		2	189.29	2873.08	6170.2
		3	193.61	2817.65	5486.13
		4	192.6	2978.09	5553.39
		5	184.33	2972.17	5626.29
		Mean	**190.07**	**2914.43**	**5662.90**
	% Stability		**96.39**	**91.09**	**93.10**

The ionization suppression of oxaceprol from plasma in mass spectrometer was too low and the matrix factor of oxaceprol and internal standard were 0.95 to 0.98 and 0.93 to 0.95 respectively **[Table-7].**

Table-7: Matrix effect (area) (N = 5) data given in mean±SD.

	Matrix effect IS		**Matrix effect Oxaceprol**	
	% of ME	**Matrix factor**	**% of ME**	**Matrix factor**
LQC (187.5/ml)	92.26±2.13	0.93±0.03	95.25±2.69	0.96±0.03
MQC (3000ng/ml)	94.99±2.44	0.95±0.02	95.43±2.19	0.95±0.02
HQC (6000ng/ml)	94.44±1.74	0.94±0.02	98.11±2.16	0.98±0.02

3.3. Pharmacokinetic study

After administration of the reference preparation, as a single dose in the fasting state, produced the maximum plasma concentration of 2905.51± 824.53 ng/ml (Cmax) at the time 2.21 + 0.72 hr (Tmax) whereas administration of the test preparation, as a single dose in the fasting state produced the maximum plasma concentration 2785.66 + 734.73 ng /ml (Cmax) at the time 2.15 + 0.65 hr (Tmax). After administration of the reference preparation, produced the area under plasma concentration-time curve (AUC 0-t) 22114.88 + 7418.59 ng.hr./ml, whereas administration of the test preparation, produced the area under the plasma concentration curve (AUC 0-t) 21622.53 + 6719.02 ng.hr./ml. When administered as a single dose, in the fasting state, the reference preparation, produced the area under the plasma concentration-time curve up to infinity (AUC 0-α) 22321.36 + 7484.97 ng.hr./ml, whereas

administration of the test preparation, produced area under the plasma concentration-time curve up to infinity (AUC 0-α) 21848.06 + 6742.61 ng.hr./ml.

Administration of the reference preparation produced the plasma elimination half-life, (T1/2) 8.48+ 1.01 hr whereas administration of the test preparation produced the plasma elimination half-life (T1/2) 8.66 + 1.76 hr. Administration of the Reference preparation, produced the plasma elimination constant (Kel) 0.083 + 0.011 hr-1, whereas administration of the test preparation produced the plasma elimination constant (Kel) 0.082 + 0.013 hr-1 **[Table-9a]** and plasma concentration pharmacokinetic curve at fasting state showed in **[Figure: 5].** The secondary pharmacokinetic parameters like AUMC (ng.hr2./ml), MRT (hr.), Cl/F (ml/hr.), Vz/F (ml), Cmax/D(ng/ml/mg), AUC0-t/D (ng.hr./ml/mg), AUC0-infy/D(ng.hr./ml/mg) of test and reference parameters showed in **[Table-9b].**

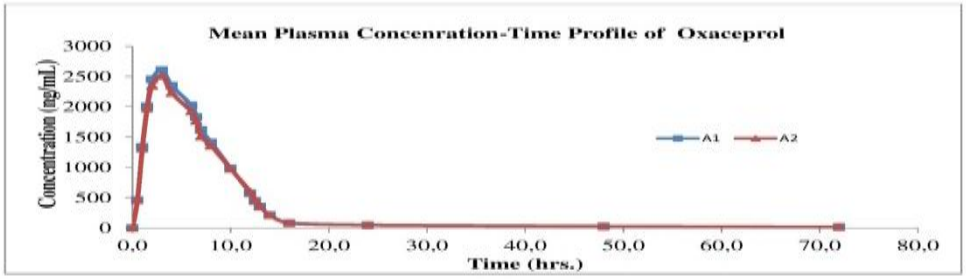
Table-9a: Mean comparative plasma pharmacokinetic parameters in n=24 volunteers of oxaceprol, data given in mean±SD.

Pharmacokinetic parameters		**OXACEPROL**	
		Reference Preparation (A1)	**Test Preparation (A2)**
C_{max} (ng./ml.)	**Mean**	2905.51	2785.66
	± S.D.	824.53	734.73
T_{max} (hr.)	**Mean**	2.21	2.15
	± S.D.	0.72	0.65
AUC 0-t (ng. hr./ml.)	**Mean**	22114.88	21622.53
	± S.D.	7418.59	6719.02
AUC 0-inf (ng. hr./ml.)	**Mean**	22321.36	21848.06
	± S.D.	7484.97	6742.61
k_{el} ($hr.^{-1}$)	**Mean**	0.083	0.082
	± S.D.	0.011	0.013
$T_{1/2}$ (hr.)	**Mean**	8.48	8.66
	± S.D.	1.01	1.76
Relative Bioavailability (%)		100 %	97.77%

Table-9b: Secondary pharmacokinetic parameters in n=24 volunteers of oxaceprol (Under fasting stage), data given in mean±SD.

Secondary Pharmacokinetic parameters	**Reference Preparation (A1)**		**Test Preparation (A2)**	
AUMC ($ng.hr^2./ml$)	**Mean**	182340.25	**Mean**	185235.75
	± S.D.	73044.43	**± S.D.**	68224.10
MRT (hr.)	**Mean**	8.14	**Mean**	8.56
	± S.D.	1.20	**± S.D.**	2.48
CL/F (ml/hr.)	**Mean**	0.03	**Mean**	0.03
	± S.D.	0.01	**± S.D.**	0.02

Vz/F (ml)	**Mean**	0.37	**Mean**	0.39
	± S.D.	0.13	**± S.D.**	0.20
C_{max}/D(ng/ml/mg)	**Mean**	4.84	**Mean**	4.64
	± S.D.	1.37	**± S.D.**	1.22
AUC_{0-t}/D (ng.hr./ml/mg)	**Mean**	36.86	**Mean**	36.04
	± S.D.	12.36	**± S.D.**	11.20
AUC_{0-infy}/D(ng.hr./ml/mg)	**Mean**	37.20	**Mean**	36.41
	± S.D.	12.47	**± S.D.**	11.24



A1- Reference Preparation
A2- Test Preparation

Figure-5: Mean comparative plasma concentration-time pharmacokinetic graph of Oxaceprol 600mg SR tablet (Under Fasting Condition)

Figure-5: Mean comparative plasma concentration-time pharmacokinetic graph of Oxaceprol 600mg SR tablet (Under Fasting Condition).

After administration of the reference preparation, as a single dose in the fed state, produced the maximum plasma concentration of 1169.39+ 324.52 ng/ml (Cmax) at the time 2.19 + 0.62 hr (Tmax) whereas administration of the test preparation, as a single dose in the fed state produced the maximum plasma concentration 1201.15 + 340.83 ng /ml (Cmax) at the time 2.19 + 0.62hr (Tmax) Administration of the reference preparation, produced the area under plasma concentration-time curve (AUC 0-t) 9673.10 + 3801.16 ng.hr./ml, whereas administration of the test preparation, produced the area under the plasma concentration curve (AUC 0-t) 9898.61+ 3506.99 ng.hr./ml. When administered as a single dose, in the fed state, the reference preparation, produced the area under plasma concentration-time curve up to infinity (AUC 0-α) 9962.92+ 3887.51 ng.hr./ml, whereas administration of the test preparation, produced area under plasma concentration-time curve up to infinity (AUC 0-α) 10190.56 + 3684.38 ng.hr./ml. Administration of the reference preparation produced the plasma elimination half-life, (T1/2) 11.88 + 1.81 hr whereas administration of the Test preparation produced the plasma elimination half-life (T1/2) 11.67 + 1.83 hr. Administration of the Reference preparation, produced the plasma elimination constant (Kel) 0.060 + 0.010 hr-1, whereas administration of the test preparation produced the plasma elimination constant

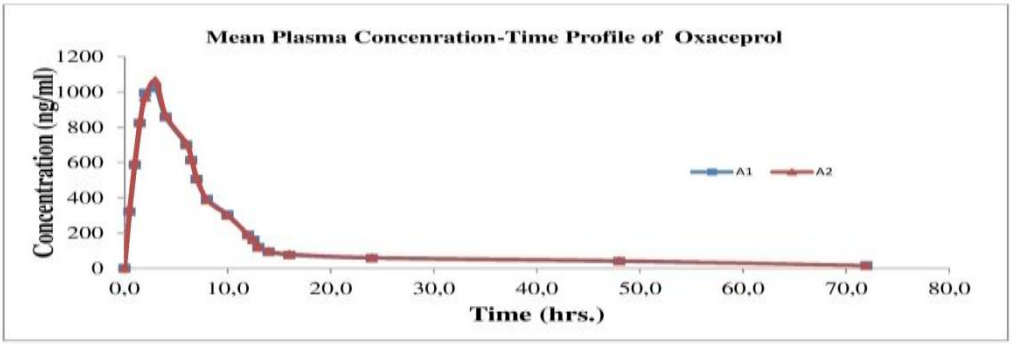
(Kel) 0.061 + 0.008 hr-1 **[Table-10a]** and plasma concentration pharmacokinetic curve at fed state showed in **[Figure: 6].** The secondary pharmacokinetic parameters like AUMC (ng.hr2./ml), MRT (hr.), Cl/F (ml/hr.), Vz/F (ml), Cmax/D(ng/ml/mg), AUC0-t/D (ng.hr./ml/mg), AUC0-infy/D(ng.hr./ml/mg) of test and reference parameters showed in **[Table-10b].**

Table-10a: Mean comparative plasma pharmacokinetic parameters in n=24 volunteers of oxaceprol, data given in mean±SD.

Pharmacokinetic parameters		**OXACEPROL**	
		Reference Preparation (A1)	**Test Preparation (A2)**
C_{max} (ng./ml.)	**Mean**	1169.39	1201.15
	± S.D.	324.52	340.83
T_{max} (hr.)	**Mean**	2.19	2.19
	± S.D.	0.62	0.62
AUC 0-t (ng. hr./ml.)	**Mean**	9673.10	9898.61
	± S.D.	3801.16	3506.99
AUC 0-inf (ng. hr./ml.)	**Mean**	9962.92	10190.56
	± S.D.	3887.51	3684.38
k_{el} ($hr.^{-1}$)	**Mean**	0.060	0.061
	± S.D.	0.010	0.008
$T_{1/2}$ (hr.)	**Mean**	11.88	11.67
	± S.D.	1.81	1.83
Relative Bioavailability (%)		100 %	102.33%

Table-10b: Secondary pharmacokinetic parameters in n=24 volunteers of oxaceprol (Under fed stage), data given in mean±SD.

Secondary Pharmacokinetic parameters	**Reference Preparation (A1)**		**Test Preparation (A2)**	
AUMC ($ng.hr^2./ml$)	**Mean**	126317.11	**Mean**	131848.39
	± S.D.	53690.74	**± S.D.**	59948.25
MRT (hr.)	**Mean**	13.08	**Mean**	13.02
	± S.D.	1.83	**± S.D.**	2.25
CL/F (ml/hr.)	**Mean**	0.07	**Mean**	0.07
	± S.D.	0.02	**± S.D.**	0.02
Vz/F (ml)	**Mean**	1.21	**Mean**	1.12
	± S.D.	0.47	**± S.D.**	0.32
C_{max}/D(ng/ml/mg)	**Mean**	1.95	**Mean**	2.00
	± S.D.	0.54	**± S.D.**	0.57
AUC_{0-t}/D (ng.hr./ml/mg)	**Mean**	16.12	**Mean**	16.50
	± S.D.	6.34	**± S.D.**	5.84
AUC_{0-infy}/D(ng.hr./ml/mg)	**Mean**	16.60	**Mean**	16.98
	± S.D.	6.48	**± S.D.**	6.14



A1- Reference Preparation
A2- Test Preparation

Figure-6: Mean comparative plasma concentration-time pharmacokinetic graph of Oxaceprol 600mg SR tablet (Under fed Condition)

Figure-6: Mean comparative plasma concentration-time pharmacokinetic graph of Oxaceprol 600mg SR tablet (Under fed Condition).

3.4. Result of statistical analysis comparative pharmacokinetic study

3.4.1. Statistical analysis in fasting stage

The results of Cmax, Tmax, Kel, T1/2, AUC0-t, AUC 0-∞ data of ANOVA for untransformed and log-transformed data observed that the parameters like Subject, Sequence, Period, and Treatment were not statistically significant at 5% level in both untransformed and log-transformed data but Subject and Sequence was statistically significant at 5% level in both untransformed and log-transformed data i.e., $P<0.05$.

The 90% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for Cmax was found to be 98.01 % to 101.21 % relative to test Preparation with reference Preparation. The 95% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for Cmax was found to be 97.68 % to 101.54% relative to test preparation with reference preparation.

The 90% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC0-t was found to be 98.34% to 101.33% relative to test Preparation with reference preparation. The 95% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC0-t was found to be 98.03% to 101.64% relative to test preparation with reference preparation. The 90% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC0-∞ was found to be 98.37 % to 101.34 % relative to test

preparation with reference preparation The 95% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC 0-∞ was found to be 98.06 % to 101.65 % relative to test preparation with reference preparation.

Application of paired t-test that the Cmax (Untransformed data) for test preparation and reference preparation was not statistically significant at 5% level and P-value = 0.531. Both the preparations are similar effects on the body.

3.4.2. Statistical analysis in fed stage

The results of Cmax, Tmax, Kel, T1/2, AUC0-t, AUC 0-∞ data of ANOVA for untransformed and log-transformed data observed that the parameters like Subject, Sequence, Period, and Treatment were not statistically significant at 5% level in both untransformed and log-transformed data but Subject and Sequence was statistically significant at 5% level in both untransformed and log-transformed data i.e., $P<0.05$.

The 90% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for C_{max} was found to be 98.83 % to 102.06 % relative to test preparation with reference preparation. The 95% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for C_{max} was found to be 98.50 % to 102.39% relative to test preparation with reference preparation.

The 90% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC0-t was found to be 98.82% to 102.01% relative to test Preparation with reference preparation. The 95% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC0-t was found to be 98.49% to 102.34% relative to test preparation with reference preparation. The 90% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC0-∞ was found to be 98.78 % to 101.94 % relative to test preparation with reference preparation The 95% confidence interval for the ratio (test/reference) of *geometric means, based on the log-transformed data for AUC 0-∞ was found to be 98.45 % to 102.27 % relative to test preparation with reference preparation.

Application of paired t-test that the Cmax (Untransformed data) for test preparation and reference preparation was not statistically significant at 5% level and P-value = 0.723. Both the preparations are similar effects on the body.

*Geometric mean was calculated as the antilog (or exponential) of the least-square means of the log-transformed data.

4. DISCUSSION

The single-dose bioequivalence study of Oxaceprol 600mg SR Tablet was conducted in 24+2 adult healthy, human, male volunteers with two preparations of Oxaceprol.

Values of C_{max}, T_{max}, and AUC $_{0-t}$, were comparable for the reference and the test preparation in the fasting state. oxaceprol was detected in plasma from 0.5 hr to 72.0 hrs for both preparations. Peak plasma levels of oxaceprol were achieved between 1.5 to 3.0 hrs for both the preparations. The mean peak plasma levels of oxaceprol with reference preparation, on the study day, ranged between 1298.03 – 4994.89 ng/ml. While the test preparation, ranged between 1073.45 – 4757.61 ng/ml. On the basis of comparison of the AUC $_{0-t}$ for oxaceprol, after single-dose administration, the relative bioavailability of the test preparation, oxaceprol 600mg SR Tablet (each film-coated sustained release tablet contains oxaceprol 600mg) was 97.77% with that of the reference preparation.

On the basis of comparison of the AUC $_{0-t}$ of oxaceprol, after single-dose administration, the relative bioavailability of the test preparation, oxaceprol 600mg SR Tablet (each film-coated sustained release tablet contains oxaceprol 600mg) was 102.33% with that of the Reference preparation, at fed condition. This is the first pharmacokinetic study of oxaceprol, in previous any literature was not reported any pharmacokinetic study of this drug.

5. CONCLUSION

On the basis of the pharmacokinetic parameters studied, it can be concluded that the test preparation, was bioequivalent with the reference preparation at fasting and fed conditions. Application of paired t-test that the C_{max} (Untransformed data) for test preparation and reference preparation was not statistically significant at 5% level and P-value = 0.531. Both the preparations were a similar effect in the body at fasting condition and application of paired t-test that the Cmax (Untransformed data) for test preparation and reference preparation was not statistically significant at 5% level and P-value = 0.723. Both the preparations were similar effects on the body at the fed condition. From the pharmacokinetic data, it was also concluded that high-fat meal food was affected the maximum concentration of oxaceprol in the human body.

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