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Bioequivalence Study of two brands of Loxoprofen tablets in Healthy Volunteers

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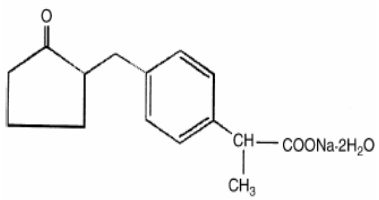
Abstract: The present study was aimed to assess the bioequivalence of two brands 60 mg tablets of loxoprofen in 12 healthy human Indian male volunteers. The study was approved by Independent Ethics Committee. After obtaining written informed consent from volunteers the study was conducted as open, randomized, two period, two treatment single dose crossover design. 5 ml of blood samples were collected at predetermined time intervals and till 24 h after administering a single dose of 60 mg loxoprofen test or reference tablet as per randomization. Plasma concentration of loxoprofen from plasma samples collected at various time points were determined by HPLC method developed and validated using UV detector. The pharmacokinetic parameters were obtained from plasma concentrations. The C_{max}, AUC_{0-t}, and AUC_{0-inf} data's were evaluated using ANOVA and it was found that no statistical significant difference observed between the test and reference formulation. The mean 90 % confidence interval for the test / reference ratios were found to be 93.13-101.87 %, 86.69-101.58 %106.34% and 86.56-100.93 % for C_{max}, AUC_{0-t} and AUC_{0-inf}, respectively and found to be within the acceptable range of 80-125 %. Based on these statistical considerations, it was concluded that the test formulation was bioequivalent to the reference formulation.

Key words: Loxoprofen sodium, bioequivalence, HPLC-UV .

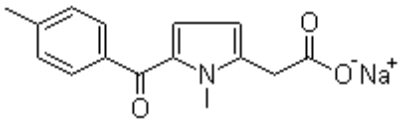
INTRODUCTION

Loxoprofen, (±)-2-[4-(2-oxocyclopentylmethyl) phenyl] propionate dihydrate, a anti-inflammatory agent has marked analgesic and antipyretic activities with relatively weak gastrointestinal ulcerogenicity [1]. Loxoprofen acts by inhibiting of prostaglandin synthesis and its pharmacological attributed to its *cis*- and *trans*-alcohol active metabolites detected after administration of loxoprofen in humans [2]. The loxoprofen molecule contains two chiral centers and is available as 4 isomers. The cyclopentanone moiety of loxoprofen enantiomer was stereo selectively reduced followed by the glucuronidation of the reduced enantiomers was considered to be the major metabolic pathway of loxoprofen in humans [3, 4]. Recently, there have been some reports indicating that NSAIDs are effective for patients with nocturia [5, 6].

Fig 1: Chemical Structure of Drug and Internal Standard



Loxoprofen sodium dihydrate



Tolmetin sodium

Araki and his associates reported that loxoprofen sodium, the most common NSAID in Japan, reduces nocturia in patients with benign prostate hypertrophy but the mechanism of this effect was not fully understood [6]. Loxoprofen is rapidly absorbed from the gastrointestinal tract and quickly converted to its active *trans*-alcohol metabolite following oral administration. The *trans*-alcohol was derived from the metabolic inversion of the enantiomers of *cis*-alcohol the metabolism of loxoprofen. It is necessary to quantify metabolites in the plasma to obtain precise information concerning the pharmacokinetics and/or pharmacodynanic characteristics of loxoprofen [7]. For comparative pharmacokinetics or bioequivalence tests, however, it might still be of practical value and generally recommended measuring the parent drug released from the dosage form rather than metabolites. The rationale of this recommendation is that the concentration-time profile of the parent drug is more sensitive to changes in formulation than a metabolite, which is more reflective of metabolite formation, distribution and elimination [8].

Literature search revealed that very few pharmacokinetic studies are published [9-16]. Although some of the reported methods are sensitive and accurate but require expensive sample preparation method like solid phase extraction and/or expensive instruments like on line column switching technique for the separation and determination of Loxoprofen [9-11], while other reported methods are less sensitive but can be used for pharmacokinetic studies. These methods require either buffer in the mobile phase which may reduce column life and/or mixture of reagent for sample preparation [12-16]. These limitations prohibit the analytical detection of loxoprofen in an analytical laboratory with limited financial and analytical instruments/equipments facilities. It was therefore felt necessary, to develop and validate a simple, rapid, sensitive and cost effective HPLC method for the determination of loxoprofen in human plasma, which can be used for the analysis of a bioequivalence study samples.

MATERIAL AND METHODS

Chemicals and Reagents

Loxoprofen sodium and tolmetin sodium were obtained from Macleods Pharmaceuticals Ltd, Mumbai, India. Acetonitrile and methanol (HPLC grade) were purchased from Qualigens Fine Chemicals, Mumbai. Ortho phosphoric acid and triethylamine (AR grade)was purchased from S.D Fine Chem. Ltd., Mumbai, India. Freshly prepared double distilled water was used throughout the study. Fresh frozen human plasma used in the method development was obtained from the National Plasma Fractionation Center, K.E.M. Hospital, Mumbai, India; and was stored at –20° C until used.

Optimized chromatographic conditions

The HPLC system consisted of a Jasco-PU980 intelligent pump (JASCO Ltd., Japan), manual injector port with 20 µl loop (Rheodyne, USA), Jasco UV–Vis 975 intelligent detector (JASCO Ltd., Japan). Separation was performed on reversed-phase column HiQ SiL C18 HS (250×4.6 mm i.d., 5µm, Japan). The mobile phase consisted of isocratic mixture of methanol : acetonitrile : 1 % triethyl amine (20:40:40, v/v). The pH of the mobile phase was adjusted to 3.0 with phosphoric acid. The mobile phase was filtered through nylon membrane filter (0.45 µm pore size, Pall, Gelman Laboratories) and ultrasonically degassed prior to use. The mobile phase was pumped at a flow rate of 1 ml/min with detection at 223 nm. The sensitivity of the detector was set at 0.001. Data were obtained and processed on Jasco-Borwin (Version 1.50) chromatography software with Hercules 2000 chromatography Interface (Version 2.0). Chromatography was carried out at ambient temperature (20-24° C). Total analysis time was 12 min.

PREPARATION OF SOLUTIONS

Preparation of working standard solution

Accurately weighed loxoprofen sodium (30.9 mg ≅ 25 mg of loxoprofen) into a 25 ml volumetric flask, was dissolved in minimum quantity of water and diluted to volume with water to furnish a 1000 µg/ml loxoprofen solution. This solution was further diluted with water to furnish 100, 10 and 1 µg/ml.

Preparation of calibration standards in plasma

Calibration standards in plasma were prepared by spiking drug free plasma with 1 ml of 1000 µg/ml loxoprofen working standard and diluted to 10 ml with drug free plasma to furnish a 100 µg/ml loxoprofen calibration standard. This solution was further diluted with drug free plasma to furnish the calibration standards of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 µg/ml. They were frozen in small portions at –20° C till analyzed.

Preparation of quality control standard solutions in plasma

Quality control standards were prepared by spiking drug free plasma with loxoprofen working standard to furnish a 'Highest Quality Control standard' (HQC) 5.0 µg/ml, 'Median Quality Control standard' (MQC) 1.0 µg/ml and 'Lowest Quality Control' standard (LQC) 0.25 µg/ml, loxoprofen quality control standards. These samples were used to validate the method and were frozen in small portions at –20° C till analyzed.

Preparation of internal standard solution

Accurately weighed tolmetin sodium (≅25 mg of tolmetin) into a 25 ml volumetric flask, dissolved in minimum quantity of water and diluted to volume with water to furnish a 1000 µg/ml tolmetin solution. This solution was further diluted with water to furnish a 100 µg/ml solution.

SAMPLE PREPARATION

To 1ml plasma sample containing loxoprofen (calibration standard), 20 µl of an internal Standard (100 µg/ml) was added and vortexed for 1 min. The drug was extracted by vortexing with 1 ml of acetonitrile for 1 min followed by centrifugation at 12000 rpm for 15 min at 4° C. 20 µl of the supernatant was injected into the column.

VALIDATION OF THE ANALYTICAL METHOD

The analytical method developed for the determination of loxoprofen from plasma was validated for its selectivity, limit of detection and quantitation, precision, accuracy, linearity, recovery, sensitivity and stability in plasma [17].

Selectivity

Six samples of drug free blank plasma sample and a quality control sample containing loxoprofen (1 µg/ml) and internal standard were extracted and analysed as described under optimized chromatographic conditions. Separation of loxoprofen, internal standard and probable impurities from plasma was checked, by comparing the chromatograms of quality control sample containing loxoprofen with that of blank plasma sample. The selectivity of the method was checked for the interference from plasma.

Linearity

Quantitative analytical results are highly influenced by the quality of the calibration curve. Linearity of the proposed method was determined by spiking various known concentrations of loxoprofen in plasma 0.1-10 µg/ml. For linearity study seven different concentrations of loxoprofen were analyzed 0.1, 0.25, 0.5, 1.0, 2.5, 5 and 10 µg/ml. To each of these samples, 20 µl of an internal standard (100 µg/ml) was added. Spiked concentrations were plotted against the peak area ratios of loxoprofen to internal standard. The best-fit line was obtained by linear regression analysis of the resultant curve. The linearity equation ($y = mx + c$) and the regression coefficient were calculated. The calibration curve requirement was set at a correlation coefficient (r^2) of 0.99 or better.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

In order to estimate LOD & LOQ, six samples of drug free blank plasma sample was extracted and analysed as described under optimized chromatographic conditions. The noise level was then determined. The LOD [Signal-to-noise ratio = 3] and LOQ for loxoprofen was determined [Signal-to-noise ratio = 10].

Precision and Accuracy

Intra–day and *inter–day* precision and accuracy were determined by analyzing quality control standards of loxoprofen (0.1, 1 and 5 µg/ml) six times a day randomly and once on each of six different days, respectively. Six samples of quality control standards of loxoprofen (0.1, 1 and 5 µg/ml) containing a fixed concentration of internal standard (20 µl of 100 µg/ml) in each of these samples were extracted and analysed as described developed under optimized chromatographic conditions and the response was measured. For acceptable *intra-day* and *inter-day* accuracy and precision should be within 85-115% and

coefficient of variation (CV) values should be <15 %, respectively.

Recovery

Recoveries were performed in triplicates on quality control standards of loxoprofen (0.1, 1 and 5 µg/ml) containing fixed amount of internal standard in each of these plasma samples. Recoveries of loxoprofen was determined by comparing the peak area ratio of loxoprofen to internal standard obtained from the extracted quality control standards with that of peak area ratio of loxoprofen to internal standard obtained from working standards solution of the respective concentration.

Stability studies

Stability of loxoprofen in plasma was tested using quality control standards for three freeze-thaw cycle and long-term stabilities. In each freeze-thaw cycle, the quality control samples were frozen at -20° C for 24 h and thawed to room temperature. The long-term stability was evaluated by storing the quality control samples at -20° C. The concentration of loxoprofen was found out on day 5, day 15 and day 30. The results were compared with those QC samples freshly prepared and the percentage concentration deviation was calculated. For the acceptance criterion of stability, the deviation compared to the freshly prepared standard should be within ±15%.

APPLICATION TO BIOEQUIVALENCE STUDY

Clinical design

The study protocol was approved by Independent Ethics Committee. Twelve healthy male Indian subjects with mean age of 21.33±1.92 years and average weight of 64.83±8.61 kg were included in the study. Subjects were excluded from the study if one of more of following criteria were present at time of medical screening, allergic to loxoprofen and other NSAIDs drugs, history or clinical data of renal or liver disease, positive test for hepatitis B, HIV, history of alcohol, drug addiction or donated blood within 72 days prior to study. Written Informed consent obtained from all the volunteers before the study was conducted. The study was conducted according to the principles outlined in the Declaration of Helsinki [18]. The study was conducted as 12 x 2 single dose, randomized, open model and complete crossover design. Volunteers were fasted overnight for 10h before the study initiated and till 4 h after drug administration. Test (60 mg of loxoprofen tablets, manufactured by Macleods Pharmaceuticals Ltd, Mumbai) and reference (60 mg LOXONIN® tablets, manufactured by Sankyo, Japan) formulations were administered per oral with 240 ml of water. Blood samples (5 ml) each were collected before dosing (0 h) and then at 10 min, 20min, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00 and 24.00 h post drug administration through an indwelling cannula into heparinised tubes. After drug administration standard breakfast and lunch were provided at 4 and 6 h. The blood samples collected at various time intervals after drug administration were immediately centrifuged at 3000 g for 15 min at 4° C, plasma was separated and stored in vials at -20° C until analysed. After a washout period of 7 days, the study was repeated in the same manner to complete the crossover design. The order of receiving the test and reference product for each subject during the two periods of the study was determined according to a randomization scheme. The plasma samples obtained at various time intervals were analysed by validated HPLC method.

Pharmacokinetic Analysis

The plasma concentration profile obtained for loxoprofen was fed into S-inverse (S-inv), computer software on BASICA® Version 1.12, to determine the pharmacokinetic parameters by non-comparatmental method. The maximum concentrations (C_{max}) and the corresponding peak time (T_{max}) were determined by checking the individual drug plasma concentration–time profiles. The elimination rate constant (K_{el}) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration-time profile. The elimination half-life ($t_{1/2}$) was calculated as 0.693/Kel. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method. The area under the curve extrapolated to infinity (AUC_{0-inf}) was calculated as $AUC_{0-t} + C_t / K_{el}$, where C_t is the last measurable concentration.

Statistical Analysis

For the purpose of bioequivalence analysis AUC_{0-t}, AUC_{0-inf} and C_{max} of loxoprofen were considered as primary variables. Bioequivalence of two formulations was assessed by means of an analysis of variance (ANOVA) for crossover design and calculating 90 % confidence interval to the ratio of test/reference using log transformed data on a Microsoft excel®. The formulation was considered bioequivalent when the difference between two compared parameters was found statistically insignificant ($p>0.05$) and confidence interval for these parameters fell within 80-125 % [19, 20].

Table 1: *Intra-Day* and *Inter-Day* Precision and Accuracy Data

Spiked Conc. (µg/ml)	Intra-day			Inter-day		
	Precision		Accuracy (%)	Precision		Accuracy (%)
	Mean±SD (n=6)	%CV		Mean±SD (n=6)	%CV	
0.25	0.232±0.014	6.034	92.80	0.232±0.013	5.567	92.67
1.0	0.907±0.042	4.592	90.67	0.913±0.055	6.030	91.33
5.0	4.880±0.193	3.952	97.60	4.947±0.227	4.598	98.933

Table 2: Recoveries of Loxoprofen and Internal Standard

Loxoprofen	Concentration (µg/ml)		
	0.25	1.0	5.0
% recovery (Mean ± SD) (n=3)	86.33±1.528	86.50±2.000	90.20±1.756
%CV	1.769	2.312	2.217
Internal Standard	**2 (µg/ml)**		
% recovery (Mean ± SD) (n=3)	78.167±1.756		
%CV	2.246		

Table 3: Stability of Loxoprofen in Human Plasma

Number of days stored	LQC (0.25µg/ml)		MQC (1µg/ml)		HQC (5µg/ml)	
	Mean ± S.D. (n = 3)	% C.V.	Mean ± S.D. (n = 3)	% C.V.	Mean ± S.D. (n = 3)	% C.V.
3-Freeze thaw studies	0.249±0.007	2.678	0.953±0.0.012	1.211	4.977±0.125	2.512
5 days	0.243±0.005	1.886	0.937±0.015	1.631	4.937±0.110	2.222
15 days	0.237±0.005	2.127	0.910±0.010	1.099	4.873±0.042	0.854
30 days	0.232±0.004	1.745	0.880±0.020	2.273	4.813±0.042	0.865

Table 4: Pharmacokinetic Parameters (Mean±SD, N=12) of Two Brands of Loxoprofen Formulations

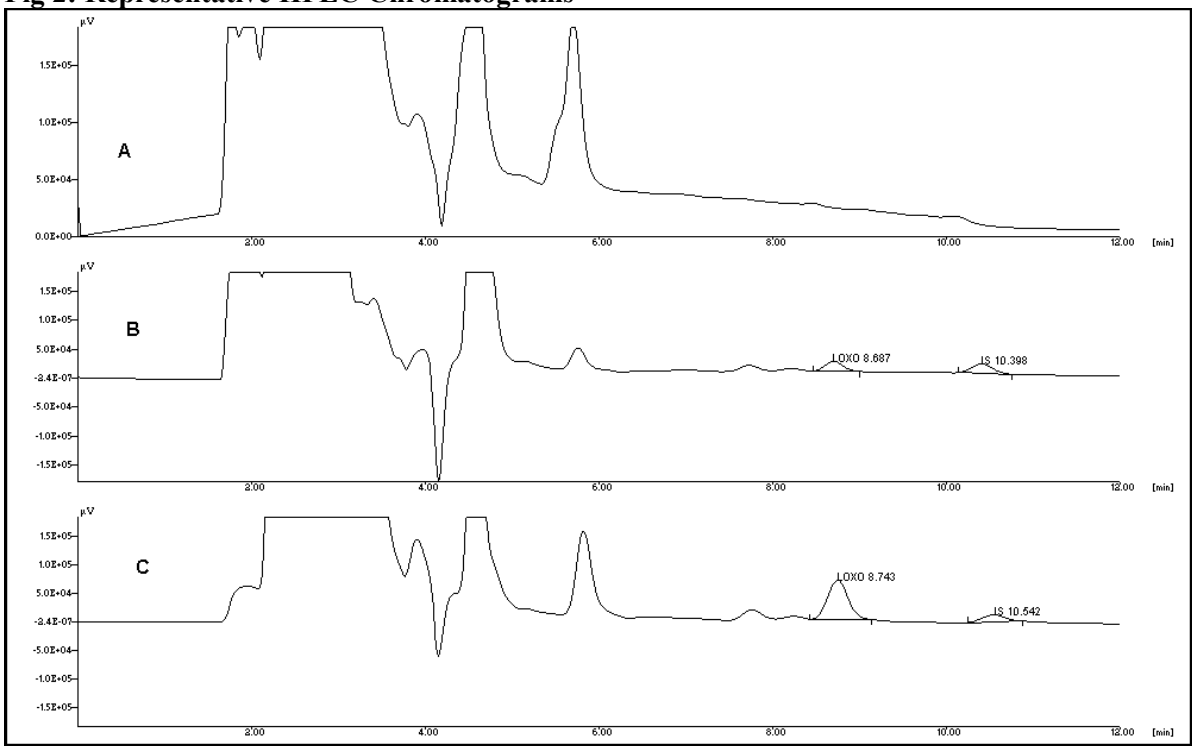
Parameters	Test	Reference	90 %CI$^{\varphi}$ (80-125 %)	P value$^{\Psi}$	T/R ratio
C_{max} (µg/ml)	6.200±0.613	6.353±0.508	93.13-101.87	0.32	97.40
T_{max} (h)	0.67± 0.18	0.54±0.17		0.07	
K_{el}	0.640±0.179	0.589±0.139		0.47	
$T_{1/2}$ (h)	1.160±0.324	1.230±0.244		0.47	
AUC_{0-t} (µg.h/ml)	9.476±1.185	10.198±2.035	86.69-101.58	0.05	93.84
AUC_{0-inf} (µg.h/ml)	9.816±1.201	10.602±2.071	86.56-100.93	0.25	93.47

Values are given as standard error of mean

($^{\Psi}$Non-significant difference at 95 % confidence limits)

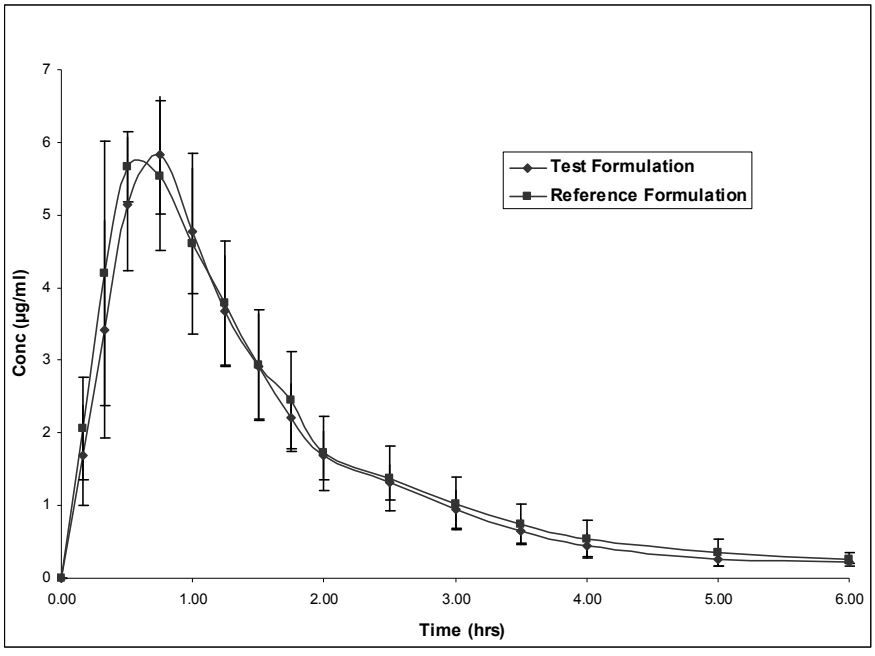
($^{\varphi}$ Statistics were applied on log-transformed data, n= 12)

Fig 2: Representative HPLC Chromatograms



(A) Blank human plasma,
(B) Calibration standard containing 1µg/ml of loxoprofen (LOXO; Rt-8.687 min) and 2 µg/ml of internal standard (IS; Rt-10.398 min) in blank human plasma and
(C) Plasma sample obtained from a volunteer 0.75 h after the oral administration of 60 mg loxoprofen test tablet- showing peaks of loxoprofen (LOXO; Rt-8.743 min) and internal standard peak (IS; Rt-10.542 min).

Fig. 3: Mean (±SD) Plasma Concentration-Time Profile of Loxoprofen 60mg Tablet in 12 Healthy Human Male Volunteers for Test and Reference Formulations



RESULTS AND DISCUSSION

The chromatographic conditions such as columns, mobile phases, flow rate, detection wavelength were studied during optimization of a method for the determination of loxoprofen from the human plasma. The optimal separation conditions of the chromatography were achieved.

Based on the literature a C18 column was the first choice in the development of chromatographic conditions. Chromatograms of four different concentrations of loxoprofen were studied on HiQ SiL C18 column with different mobile phase compositions. Due to its unionized state in acidic conditions, mobile phase with acidic pH was preferred. Initially a mobile phase consisting of acetonitrile : methanol : water in the proportion of 40:20:40 (v/v), pH of the mobile phase adjusted to 3 with ortho phosphoric acid was tried and found that the peak of loxoprofen showed tailing. Water was replaced with potassium dihydrogen phosphate buffer (pH 3). The mobile phase consisting of acetonitrile : methanol : phosphate buffer (pH 3) in the proportion of 40:20:40 (v/v), was tried and found that an impurity from the plasma sample was eluted with loxoprofen, tailing in loxoprofen peak and the resolution between the loxoprofen and IS was also found to be less. The mobile phase was modified to acetonitrile : methanol : 1 % triethylamine in the proportion of 40:20:40 (v/v), pH of the mobile phase adjusted to 3 with ortho phosphoric acid delivered at a flow rate of 1 ml/min with detection at 223 nm not only solved the problem of selectivity and tailing of loxoprofen but also resolution between loxoprofen and IS and was finally adopted.

For internal standard, drugs which have similar solubility properties as loxoprofen and can be resolved from the analyte and other plasma impurities were chosen during method development. Drugs like dexibuprofen, torsemide and tolmetin sodium were attempted for selection as internal standard. The drugs except tolmetin sodium tried were found to be either overlapping with retention time of loxoprofen (torsemide) or the retention time was too high (dexibuprofen) under the optimized chromatographic conditions. Loxoprofen and tolmetin sodium (IS) showed good resolution with retention times of 8.592±0.5 min and 10.3±0.5 min, respectively, under optimized chromatographic conditions. Hence, tolmetin sodium was selected as an internal standard.

The molecular structures of loxoprofen sodium and IS (tolmetin sodium) are shown in Fig. 1. The chromatograms of blank plasma, working standard solution, blank plasma spiked with 1 µg/ml of loxoprofen and 2 µg/ml of IS and a volunteer's plasma at 0.75 h after the oral administration test formulation are shown in Fig. 2. There are no apparent plasma components which interfered with the peaks corresponding to loxoprofen and IS.

Calibration curves obtained by analyzing quality control plasma samples spiked with various amounts of authentic loxoprofen (0.1-10 µg/ml.) showed good linearity ($Y=0.7356x+0.0814$, $r^2=0.9984$). The LOQ of Loxoprofen in plasma was verified as 0.1 µg/ml. This was the lowest concentration at which the accuracy was between 80-120 % and precision was within 20 %. The LOD was 0.05 µg/ml at a signal-to-noise ratio of 3. The results for accuracy and precision for loxoprofen quality control standards are presented in table 1. All the values of accuracy and precision including LOQ fell within the limits considered as acceptable. The recovery of loxoprofen and IS from 1 ml of plasma was measured for the quality control samples are tabulated in table 2. The stability studies for three freeze-thaw cycles appeared to have no effect on stability of the loxoprofen and quality control samples of loxoprofen stored in a freezer at -20° C remained stable for at least one month. These studies suggested that human plasma samples containing loxoprofen can be handled under normal laboratory conditions without significant loss of compound (table 3).

This method has been successfully applied for a bioequivalence studies after single oral administration of two brands of loxoprofen (60 mg) tablets in 12 healthy male volunteers. Plasma profiles of loxoprofen concentration versus time after the oral administration of a single dose of both formulations exhibits closely similar patterns, which were nearly superimposable (Fig. 6). At the first sampling time (10 min) loxoprofen appeared in plasma in both groups. Pharmacokinetic parameters (table 4) of both formulations such as $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max}, $t_{1/2}$ and K_{el} were comparable to the corresponding parameters obtained after a single oral dose of 60 mg loxoprofen as reported by earlier literature. The ratio of test/reference (T/R) and 90% percent confidence intervals (90 CIs) for the observed pharmacokinetic parameters after an oral administration of 60 mg loxoprofen tablets were within the range of 80-125% in accordance with the Food and Drug Administration Bioequivalence Guideline [20] and are presented in table 4. There were no statistical differences observed between test formulation and reference formulation of loxoprofen using ANOVA. The bioequivalence study of loxoprofen were carried for the first time in the Indian healthy human male volunteers as no pharmacokinetic

data was available in Indian male volunteers. Hence the studies were conducted for 24 h to get complete description of concentration-time profile in the Indian healthy male volunteers. However after 6 h; loxoprofen was not detectable in the plasma sample by UV detector. The half- life of loxoprofen from the study was about 1-1.5 h. For comparing the bioequivalence of two products, 3 half-life data of drug is sufficient and which is about 3-4.5 h. Hence, the developed method is sensitive enough to apply for bioequivalence study of 60 mg loxoprofen tablets.

CONCLUSION

An HPLC-UV based method has been developed for quantification of loxoprofen in human plasma. The present method is very simple, rapid and readily applicable to pharmacokinetic and routine bioequivalence studies of these compounds with an acceptable sensitivity. Based on the statistical results, it is concluded that the test formulation is bioequivalent to the reference formulation and that both products can be considered alternative in the clinical situation.

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REFERENCES

1. Terada A., Naruto S., Wachi K., Tanaka Y., Iizuka Y., and Masaka E., Synthesis and antiinflammatory activity of [(cycloalkylmethyl)phenyl]acetic acids and related compounds, J.Med.Chem., 1984, 27, 212-216.
2. Sugimoto M., Kojima T., Asami M., Iizuka Y. and Matsuda K., Inhibition of prostaglandin production in the inflammatory tissue by loxoprofen-Na, an anti-inflammatory prodrug. Biochem. Pharmacol., 1991, 42, 2363-2368.
3. Matsuda K., Tanaka Y., Ushiyama S., Ohnishi K. and Yamazaki M, Inhibition of prostaglandin synthesis by sodium 2-[4-(2-oxocyclopentylmethyl)phenyl] propionate dihydrate (CS-600), a new anti-inflammatory drug, and its active metabolite in vitro and in vivo. Biochem Pharmacol., 1984, 33, 2473-2478.
4. Naruto S., Tanaka Y., Hayashi R. and Terada A., Structural determination of rat urinary metabolites of sodium 2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (loxoprofen sodium), a new anti-inflammatory agent. Chem Pharm Bull (Tokyo)., 1984, 32, 258-267.
5. Le Fanu J., The values of aspirin in controlling the symptoms of nocturnal polyuria. BJU Int., 2001, 88, 126-127.
6. Araki T., Yokoyama Y. and Kumon H., Effectiveness of nonsteroidal anti-inflamrnatory drug for nocturia on patients with benign prostatic hyperplasia: a prospective non-randomized study of loxoprofen sodium 60 mg once daily before sleeping. Acta Med Okayama., 2004, 58, 45-49.
7. Tanaka Y., Nishikawa Y. and Flayashi R., Species differences in metabolism of sodium 2-[4-(2-oxocyclopentylmethyl)-phenyl]propioniate dihydrate (loxoprofen sodium), a new anti-inflammatory agent. Chem Pharm Bull (Tokyo)., 1983, 31, 3656-3664.
8. Midha K.K., Rawson, M.I. and Hubbard J.W., The role of metabolites in bioequivalence. Pharm Res. 2004, 21, 1331-1344.
9. Kanazawa H., Tsubayashi A., Nagata Y., Matsushima Y., Mori C., Kizu J. and Higaki M., Stereospecific analysis of loxoprofen in plasma by chiral column liquid chromatography with a circular dichroism-based detector. J Chromatogr A., 2002, 948, 303-308.
10. Hirai T., Matsumoto S. and Kishi I., Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. J Chromatogr B., 1997, 692, 375-388.
11. Cho H.Y., Park C.H. and Lee Y.B., Direct and simultaneous analysis of loxoprofen and its diastereometric alcohol metabolites in human serum by on-line column switching liquid chromatography and its application to a pharmacokinetic study. J Chromatogr B., 2006, 835, 27-34.
12. Jhee O.H., Lee M.H., Shaw L.M., Lee S.E., Park J.H. and Kang J.S., Pharmacokinetics and Bioequivalence Study of Two Brands of Loxoprofen Tablets in Healthy Volunteers. Arzneimittelforschung, 2007, 57, 542-546.
13. Koo T.S., Kim D.H., Ahn S.H., Kim K.P., Kim I.W., Seo S.Y., Suh Y.G., Kim D.D., Shim C.K.

and Chung S.J., Comparison of Pharmacokinetics of Loxoprofen and its Active Metabolites after an Intravenous, Intramuscular and Oral Administration of Loxoprofen in Rats: Evidence for Extrahepatic Metabolism. J Pharm Sci. 2005, 94, 2187-2197.

14. Kim I.W., Chung S.J. and Shim C.K., Altered metabolism of Orally Administrated loxoprofen in Human Subjects after an Oral Administration of Loxoprofen for Three Consecutive Days Followed by a Seven-Day Washout. J Pharm Sci. 2002, 91, 973-979.
15. Choo K.S., Kim I.W., Jung J.K., Suh Y.G., Chung S.J., Lee M.H. and Shim C.K., Simultaneous determination of loxoprofen and its diastereomeric alcohol metabolites in human plasma and urine by a simple HPLC-UV detection method. J Pharm Biomed Anal. 2001, 25, 639-650.
16. Kim I.W., Choo K.S., Han T.G., Kim K.S., Chung S.J., Lee M.H. and Shim C.K., Decreased oral bioavailability of loxoprofen at second administration in human subjects. Int J Clin Pharmacol Ther. 2000, 1, 10-14.
17. Center for Drug Evaluation and Research (CDER). 2001. Guidance for Industry, Bioanalytical Method Validation. Department of Health and Human Services. Food and Drug Administration Rockville, Maryland: U.S.

    http://www.fda.gov/cder/guidance/index.html
18. ICH Harmonized Tripartite Guideline for Good Clinical Practice. 1996. Brookwoood Medical Publication Ltd. Surrey, UK. 1-45.
19. Center for Drug Evaluation and Research (CDER). 2001. Guidance for Industry, Statistical Approaches in Establishing Bioequivalence. U.S. Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Rockville, Maryland: U.S.

    http://www.fda.gov/cder/guidance/index.html
20. Central Drug Standard Control Organization, (CDSCO). 2005. Schedule Y (Amended version). Directorate General of health Services, Ministry of Health and Family Welfare, Govt. of India. www.cdsco.nic.in.
21. Pharmacopoeial Forum. 1993, May. US Department of Health and Human Service, Food and Drug Administration.

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