

resolution of pistacienoic acids with symmetrical and reproducible peaks was achieved by using chloroform:methanol (95:05) by HPTLC while for HPLC the mobile phase consisting of acetonitrile:water:phosphoric acid, (80:20:1) gave satisfactory separation and resolution. Using the proposed HPTLC methods, the migration distance of pistacienoic acids was about 37 mm while the retention time using HPLC was 4.8 min.

The calibration curves for HPTLC and HPLC were linear in the range of 1.0 to 8.0 µg and 1.25 to 10.0 µg, respectively. Further, the correlation coefficient 0.999 and 0.998 indicates good linearity between concentration and area for both the methods. The methods allow reliable quantification of pistacienoic acids and provide good resolution and separation of marker compound from other constituents of *P. integerrima*. Further recovery values of 97.56% - 99.37% (98.55% ± 0.92%) and 98.59% - 100.17% (99.10 ± 0.92%) were obtained using HPTLC and HPLC, showing excellent

reliability and reproducibility of proposed methods. The values of pistacienoic acids were observed to be 3.46% and 3.68% using HPTLC and HPLC, respectively. The proposed HPTLC and HPLC methods are rapid, simple and accurate for quantitative monitoring of pistacienoic acids in *P. integerrima* and can be used for routine quality testing.

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Bioequivalence Study of Rosiglitazone Maleate 2 mg Tablets on Twelve Indian Human Male Volunteers

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Accepted 24 February 2002

Revised 11 February 2002

Received 3 May 2001

Rosiglitazone is a second-generation aminopyridyl thiazolidinedione that has been observed to be a safe and effective oral glucose-lowering agent in diabetic patients. In the present study two different preparations containing 2 mg rosiglitazone were compared in twelve healthy Indian human male volunteers under fasting condition. The trial was performed in a randomized two-way crossover design with a wash out period of one week. Rosiglitazone was extracted from human plasma using liquid-liquid method of extraction. HPLC method was used to determine the plasma concentration of the drug.

Rosiglitazone is a second-generation aminopyridyl thiazolidinedione that has been observed to be a safe and effective oral glucose-lowering agent in diabetics¹. This class of compounds has been demonstrated to lower plasma glu-

cose by reducing insulin resistance and increasing peripheral glucose disposal^{2,3}. The present study was performed to investigate bioequivalence between two preparations of 2 mg rosiglitazone maleate tablets. In order to analyze plasma samples a HPLC method was developed. This newly developed method was first validated^{4,5}. The proposed

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method, consists of a single step extraction of rosiglitazone followed by HPLC with UV detection. Lowest limit of quantitation was found to be 15 ng/ml.

Administration of standard formulation (Avandia 2 mg) manufactured by Smithkline Beecham (USA) showed a maximum concentration of rosiglitazone (mean \pm S.E.) 215.9 ± 9.2 ng/ml in plasma at 1.21 ± 0.21 h, while that of test formulation (rosiglitazone maleate 2 mg tablet) manufactured by Sun Pharmaceutical Industries showed a maximum concentration of rosiglitazone, 213.3 ± 24.1 ng/ml at 1.48 ± 0.24 h.

In this bioequivalence study the test preparation used was manufactured by Sun Pharmaceutical Industries, which was compared with the reference preparation (Avandia 2 mg) internationally marketed innovator brand. Working standard was supplied by Sun Pharmaceutical Industries with percentage purity of 99.92 % on dried basis. Acetonitrile, methanol and methylene chloride used were of HPLC grade manufactured by Spectrochem Pvt. Ltd. HCl and ammonium acetate were AR grade manufactured by E. Merck (India) Ltd. and Qualigens Fine Chemicals, respectively.

A Jasco PU 980 Intelligent pump fitted with 50 μ l loop and Jasco UV-970 UV/Vis detector were used. The recorder used was Borwin software version 1.21. The chromatography was carried out on C_{18} (150 mm X 4.6 mm) with particle size of 5 μ m column using acetonitrile:0.01 M ammonium acetate buffer in the volume ratio of 40:60 pH adjusted to 7.00 ± 0.05 with triethylamine at a flow rate of 1.0 ml/min. The detection was carried out using a UV/Vis detector at 247 nm.

Stock solution of rosiglitazone was prepared by weighing 0.0331 g of rosiglitazone maleate (99.92%), which was then dissolved in methanol to get 1000 μ g/ml of rosiglitazone. Further dilutions of the standard solution were done in mobile phase. Fixed volume of the drug solutions was spiked from standard solution of different concentration to get the desired linearity level in human plasma.

One millilitre of plasma was taken in a stoppered glass tube. To it, 0.2 ml of 0.1 N HCl solution was added and vortexed for 30 s. Ten millilitres of methylene chloride were added and the tubes were shaken for 10 min. After shaking, the tubes were centrifuged for 10 min at 850 rpm, 8 ml of organic layer was separated and evaporated to dryness on low volume evaporator under nitrogen stream at 48° for 15 min. The residue was reconstituted in 0.10 ml of mobile phase

and 50 μ l of this was into the chromatographic system. Calibration curves were obtained by plotting the peak area values of the rosiglitazone against the concentration over the range of 15 to 300 ng/ml.

Detector response for rosiglitazone was found to be linear in the range of 15-300 ng/ml. The linearity of the calibration curve is determined by a unweighted least square regression analysis by measuring the peak area value for the drug peak. The correlation coefficient for rosiglitazone varied from 0.995 to 0.999. Percentage nominal for the back calculated concentration was between 80 to 120% for the lowest level of the calibration curve and 85 to 115% for the other levels. Coefficient of variation was less than 20% for the different levels of the linearity. Lowest limit of detection was found to be 10 ng/ml and lowest limit of quantification was found to be 15 ng/ml.

Between-run precision results (% C.V.) ranged between 6.52 to 9.55%, Between-run accuracy (% nominal) results obtained at the low, medium and high quality control samples (QCs) were 105.15, 99.43 and 99.12% respectively. The within-run precision and accuracy were determined from total of 5 replicates of the low, medium and high QCs. Within-run precision results (% C.V.) ranged between 1.00 to 7.65%. Within-run accuracy (% nominal) results obtained at the low, medium and high QCs were 109.59, 95.96 and 98.05% respectively. Freeze thaw stability evaluation involved analysis of low, middle and high quality control samples. The samples were found to be stable for three freeze thaw cycles⁵.

Before commencing the bioequivalence study, the protocol of this study was approved by IRB (Institution Review Board). Twelve healthy human Indian male subjects were included in the study. Before enrolling them for the study, routine hematological, biochemical and pathological tests of these volunteers were performed. Only healthy human male volunteers were included between the ages of 18-40 years. The study was performed according to cGCP and according to the declaration of Helsinki. All the subjects included in the study had given the written consent for their participation. The subjects were also completely informed about their rights, especially about right of withdrawing from the study without any explanation.

The study was designed as a single dose, randomised, two way crossover design. Each volunteer participating in the study received one of the two formulations on two different days with a wash out period of one week. Volunteers

TABLE 1: PHARMACOKINETIC PARAMETERS OF ROSIGLITAZONE MALEATE TABLETS.

Parameters	Standard \pm S.D.	Test \pm S.D.	% Ratio (Test/Standard)	Limits of 90% confidence interval
C_{max} (ng/ml)	215.9 \pm 31.7	213.3 \pm 83.5	98.80	80.74-116.85
In C_{max}	5.3644 \pm 0.1502	5.3054 \pm 0.3386	101.46	84.00-118.93
$AUC_{(0-t)}$ (ng/ml x h)	1367.45 \pm 518.57	1387.45 \pm 422.67	102.40	85.30-119.50
In $AUC_{(0-t)}$	7.1580 \pm 0.3656	7.1854 \pm 0.3488	94.27	80.12-110.91
$AUC_{(0-\infty)}$ (ng/ml x h)	1441.89 \pm 555.27	1476.53 \pm 419.49	102.78	86.19-122.57
In $AUC_{(0-\infty)}$	7.2117 \pm 0.3593	7.2548 \pm 0.3196	104.40	88.47-123.20
T_{max} (h)	1.21 \pm 0.71	1.48 \pm 0.84	NA	NA
$T_{1/2}$ (h)	3.63 \pm 1.98	4.18 \pm 1.33	NA	NA
K_{el} (h ⁻¹)	0.249 \pm 0.135	0.180 \pm 0.053	NA	NA

SD stands for standard deviation while NA denotes not applicable.

fasted for 10 h prior to the drug administration. One tablet of either formulation was administered with 240 ml of water. Blood was collected in centrifuge tubes containing 0.1 ml of 10% EDTA. Postdose sampling times after drug administration were 0.25, 0.50, 0.75, 1, 1.25, 1.50, 2, 3, 4, 6, 8, 10, 12 and 24 h. Blood samples were centrifuged immediately, plasma was separated and stored frozen at $-20 \pm 5.0^\circ$ with appropriate labels identifying subject numbers, study day and time of blood collection.

Plasma concentration of the unknown samples was calculated on the basis of linearity levels injected along with each chromatographic run⁵. Quality control samples were also the part of instudy validation⁵. Based on this concentrations various pharmacokinetic parameters were calculated. The peak plasma concentration (C_{max}) and time to peak

concentration (t_{max}) were obtained from the plasma concentration-time data. The $AUC_{(0-t)}$ values were calculated by linear trapezoidal method, K_{el} , $t_{1/2}$ and $AUC_{(0-\infty)}$ were calculated using Winnonlin software developed by Pharsight Corporation, California (USA). This software is validated and is accepted by the regulatory bodies. The pharmacokinetic parameters for the test formulation were compared with those of reference formulation using a student paired t-test and ANOVA. No adverse effects were observed during both the runs of the study.

For this standardized chromatographic conditions, retention time of rosiglitazone was found to be 8.7 min. Percentage extraction yield (absolute recovery) was calculated at two levels i.e. at 30 and 250 ng/ml by comparing the peak area values of plasma extracted levels and unextracted standards. Mean percentage extraction yield was found to be 66.15%. Proposed HPLC assay method provides reproducible estimates of the drug concentration in volunteer plasma with sufficient sensitivities to allow pharmacokinetic and bioequivalence study.

The power of test for the C_{max} , $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values was 97.44%, respectively. For the log-transformed data it was 91.15%. ANOVA was applied to various pharmacokinetic parameters with subject, period and treatment as variables. When $AUC_{(0-t)}$ of both formulations were compared, test preparation rosiglitazone maleate 2 mg tablets of Sun Pharmaceutical Industries showed a relative bioavailability of 101.46%. Based on the untransformed data and log transformed data of C_{max} , In C_{max} , T_{max} , $AUC_{(0-t)}$, In $AUC_{(0-t)}$, $AUC_{(0-\infty)}$

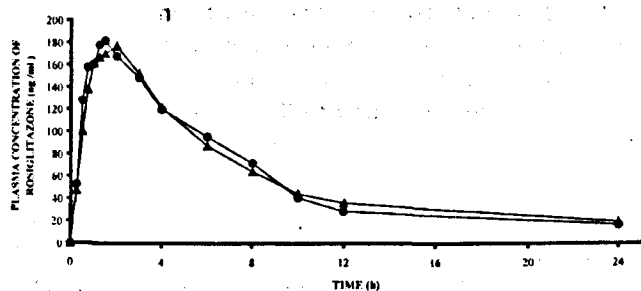


Fig. 1: Plasma concentration vs. time curve.

Mean plasma concentration (ng/ml) vs. time (h) after administration of rosiglitazone (2 mg) test formulation (- Δ -) or standard formulation (- \bullet -) to 12 healthy human male volunteers.

...), In $AUC_{(0-\infty)}$ at 90% confidence interval both the formulations were found to be bioequivalent. For the present test preparation all the acceptance criteria were completely fulfilled as per the guidance of CPMP for bioequivalence studies i.e 0.80 to 1.20 for untransformed data and 0.80 to 1.25 for the logtransformed data. Thus, bioequivalence between the test and standard preparation was demonstrated.

ACKNOWLEDGEMENTS

We are thankful to Sun Pharmaceutical Industries for providing us with the working standard and sponsoring the bioequivalence study. One of the authors, A.A. Moghe, is thankful to University of Mumbai for granting Pandit Bhagwandin Dube and Ramdulari Dube Scholarship in Science and Sir Currimbhoy Ebrahim and bai K.N.J.P. Scholarship.

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Inclusion Complexation of Meloxicam with β -Cyclodextrin

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Accepted 27 February 2002

Revised 19 February 2002

Received 14 November 2000

Inclusion complexes of meloxicam with β -cyclodextrin (β -CD) were prepared by various methods like grinding, kneading, solid dispersion and freeze drying. The prepared complexes were evaluated by FTIR, X-ray diffraction, differential scanning calorimetry and scanning electron microscopy. The *in vitro* dissolution rate of drug- β -CD complex was faster compared to the drug alone.

Cyclodextrins are cyclic maltooligosaccharides which have been extensively used to increase aqueous solubility of poorly soluble drugs^{1,2}. Amongst the existing cyclodextrins, β -cyclodextrin (β -CD) has been used extensively to modify their physico-chemical properties^{3,4}. Meloxicam is a preferential cox-2 inhibitor which is used in the treatment of os-

teoarthritis and rheumatoid arthritis^{5,6}. The major drawback of this drug is its low aqueous solubility which delays its absorption from GI tract and its prolonged use is associated with incidence of side effects that include GI perforations, ulcerations and bleeding. Therefore, an attempt has been made to improve the aqueous solubility of meloxicam by complexing it with β -CD.

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