

## BIOEQUIVALENCE OF TWO CAPSULE FORMULATIONS OF PIROXICAM

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*This study presents the results of two treatment randomized crossover investigation of 20 healthy male volunteers to assess the bioequivalence of two products of piroxicam (Feldene<sup>R</sup> and Unicom<sup>R</sup> capsules) using an HPLC method developed specifically for quantifying piroxicam in serum. Both products were administered as a single oral dose (1 x 20 mg capsule) separated by a two weeks washout period. The results of this investigation indicated that there were no statistically significant differences between the two products in the mean concentration-time profiles. With the exception of the  $T_{max}$  parameter, which was significantly shorter for Unicom<sup>R</sup> compared to Feldene<sup>R</sup>, no statistically significant differences were observed between the products for the derived pharmacokinetic parameters, including  $AUC_{0-24h}$ ,  $AUC_{0-144h}$ ,  $AUC_{0-inf}$ ,  $T_{lag}$ ,  $C_{max}$ ,  $K_e$ , and  $T_{1/2e}$ . Concerning the relative extent of absorption, assessed by the AUC ratio (Unicom/Feldene) for different time intervals (24 hours, 144 hours, and infinity), the average values with their 95% confidence limits (C.L.) were respectively  $0.98 \pm 0.04 (0.90-1.06)$ ,  $1.02 \pm 0.06 (0.90-1.14)$ , and  $1.02 \pm 0.05 (0.92-1.12)$ . These findings clearly indicate that the two products are bioequivalent in the extent of drug absorption.*

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**Keywords :** Piroxicam, Bioequivalency, HPLC Analysis, Pharmacokinetic Parameters.

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### Introduction

Piroxicam (4 - hydroxy - 2 - methyl - N - (2 - pyridinyl) - 2H - 1,2 - benzothiazine - 3-carboxamide - 1,1 - dioxide), an enolic acid compound with a pka of 6.3, is a non-steroidal anti-inflammatory drug used for the treatment of several rheumatic and inflammatory disorders including rheumatoid arthritis and osteoarthritis (1-4).

The pharmacokinetics of piroxicam has been reviewed by Malden Mihalic et al (5). This drug is readily absorbed after oral administration and is prone to accumulation after repeated doses, reaching steady state levels after about 7 days. In man, it is extensively metabolized to apparently inactive metabolites mainly *via* hydroxylation of pyridyl ring in the para position, followed by conjugation with glucuronic acid (6). About 10% of a single oral dose of piroxicam is excreted unchanged in urine within the first 10 days following administration. Piroxicam has a half-life of about 40 hours following a single dose administration. Due to the extended plasma half-life of pi-

roxicam, plasma concentration remains apparently over the next 24-48 hours. Plasma concentrations are roughly related to the administered dose. It has been shown that 10 and 100 mg doses yielded respectively 0.85 and 13.5  $\mu\text{g/ml}$  after a single dose. At concentrations ranging between 5-30  $\mu\text{g/ml}$ , piroxicam is 99.3% bound to plasma proteins. Thus, it might be expected to displace other highly protein bound drugs. The drug penetrates into the synovial fluid of patients with rheumatoid arthritis and attains concentrations of about 40% of that in the plasma (7,8).

The objective of this study was to compare the pharmacokinetic behavior and concentration-time profiles of two capsule formulations of piroxicam; Unicom<sup>R</sup> (a test product) in the form of 20 mg capsule and Feldene<sup>R</sup> (a reference product) in the form of 20 mg capsule.

In this paper, a high performance liquid chromatographic (HPLC) assay for tenoxicam and piroxicam developed by M. Salem et al was used (9).

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\* Correspondence

## Subjects and Methods

### Subjects

Subjects included in this study were 20 healthy adult male volunteers ranging in age from 19 to 36 years ( $27.6 \pm 1.1$  years), with mean body weight,  $75.6 \pm 2.0$  kg (55 to 90 kg) and height,  $171.4 \pm 1.3$  cm (161 to 180 cm). On the basis of medical history, clinical examination, and laboratory investigations (hematology, blood biochemistry, and urine analysis), none of the participants had revealed any medical abnormality. Signed informed written consent was obtained from the volunteers prior to the enrollment in the study.

### Products

- A : Feldene<sup>R</sup> capsule (produced by pfizer, U.S.A.) containing 20 mg of piroxicam (Batch # 31410 C)
- B : Unicom<sup>R</sup> capsule (produced by United Pharmaceutical Company) containing 20 mg of piroxicam (Batch # 40437)

### Treatment design and doses

Each subject received the two products (A and B) in two treatment days with a 14 day washout interval. The order of product administration was done according to a randomized cross-over design taking in consideration that equal number of subjects receive each product at both phases. A dose of 20 mg (1 x 20 mg capsule) of either product (Feldene<sup>R</sup> "A" or Unicom<sup>R</sup> "B") was administered in each phase.

Subjects fasted for at least 10 hours prior to drug administration and for five hours afterwards. Standard meals were served at 4, 10 and 23 hours following drug dosing. Cigarettes and beverages containing xanthines were not allowed for 12 hours prior to drug administration and 24 hours post drug administration. Blood samples (10 ml) were collected at 0 (predose), 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 18.0, 24.0, 48.0, 72.0, 96.0, 120 and 144 hours post dose. Following blood clotting, serum samples were separated after centrifugation and kept frozen at -10°C until assayed.

### Analytical technique

#### Calibration curve data and Sample preparation for HPLC assay

A calibration standard in 0.5 ml blank plasma was prepared to give 0 (no piroxicam added), 40, 100, 200, 500, 1000, 2000, 3000 ng/ml concentrations. A 75  $\mu$ l aliquot of internal standard (Tenoxicam) (5  $\mu$ g/ml), and 0.25 ml of phosphate buffer pH 2.0 was added to the mixture which was shaken on a vortex mixer for 30 sec. 6 ml of ethylacetate was added. The mixture was shaken again on a vortex for 2 min., and centrifuged for 5 min. at 3000 r.p.m. The supernatant was transferred

to 10 ml tube and evaporated to dryness at 50°C in a water bath under a stream of dry nitrogen. The residue was reconstituted in 250  $\mu$ l of water: acetonitrile: acetic acid (48:50 2% V:V) vortex mixed for 30 sec. and transferred to a disposable polypropylene micro-centrifuge tube (1.5 ml Eppendorf) and centrifuged for 2 min. at 11500 r.p.m. An appropriate aliquot was then injected directly into the injector. Plasma samples of the volunteers were processed for analysis in an identical manner. Peak height ratios (piroxicam/internal standard) were measured and plotted against concentration of piroxicam.

### Chromatographic conditions

All analysis were performed with an HPLC system consisting of Beckman 144 M solvent delivery system, a variable wavelength U.V detector (JASCO 375 UV) and a rheodyne injector. (7125, fitted with 100  $\mu$ l loop). The column used was a Lichrospher 100 Rb- 18, 5 micrometer column, 250 x 4 mm (Shandon, Germany). The mobile phase consisted of 67% phosphate buffer, 23% acetonitrile and 10% methanol. The pH of the mobile phase was adjusted to 7.3 with concentrated potassium hydroxide solution. The flow rate of the mobile phase was 1 ml/min. and the detector wavelength was set at 360 nm and at a sensitivity of 0.01 a.u.f.

### Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using the PKCALC computer program. The elimination rate constant ( $K_e$ ) and the half-life of elimination ( $T_{1/2e}$ ) were calculated by linear regression of the terminal slope of the serum concentration-time profiles. Areas under the concentration-time curves for 24 hours ( $AUC_{0-24h}$ ) and for 144 hrs ( $AUC_{0-144h}$ ) were estimated by the hybrid logarithmic/linear trapezoidal rule, and AUC for infinity ( $AUC_{0-inf}$ ) was obtained by adding  $C/K_e$  to  $AUC_{0-144h}$ , where C is the last measured concentration. Other pharmacokinetic parameters, such as  $T_{lag}$ ,  $T_{max}$  and  $C_{max}$  were estimated by inspection of the concentration-time curves.

### Statistical analysis

All results are expressed as mean  $\pm$  s.e.m., and variability about the mean is expressed as the coefficient of variation (c.v.). In addition, the 95% confidence limits were calculated for all the parameters and for the AUC ratio of product B (Unicom<sup>R</sup>) to product A (Feldene<sup>R</sup>). Statistical analysis on log-transformed data was performed by two way analysis of variance to test for drug and phase effects. Differences between the means were considered statistically significant for P values equals to or less than 0.05.

## Results and Discussion

Serum concentration of piroxicam was determined by a specific and sensitive HPLC method. Typical chromatograms of a serum

sample collected from a volunteer and the corresponding blank serum are presented in Fig.1. The analytical procedure was validated by determination of its specificity, linearity, and reproducibility. Specificity was confirmed by the lack of interference from endogenous constituents and from some of the commonly used drugs, such as theophylline, caffeine, ampicillin, famotidine, chlorpheniramine

maleate, paracetamol, phenylephrine hydrochloride, ranitidine hydrochloride, terfenadine, metochlopramide, and indomethacin. Linearity was assessed by evaluating the mean standard deviation from ten calibration curve data ( $r = 0.9988 \pm 7.93 \times 10^{-4}$ , slope (B) =  $0.6929 \pm 3.63 \times 10^{-2}$ , intercept (A) =  $2.43 \times 10^{-2} \pm 8.3 \times 10^{-3}$ ). The within-day precision was evaluated by 6 replicate analysis of pooled-plasma samples containing piroxicam at three different concentrations. The coefficient of variation ranged from 2.32-5.25%. The between-day precision was similarly evaluated on several days up to 2 weeks with values ranging between 2.51-6.63% (Table 1).

The mean serum concentrations of piroxicam following oral administration of the two products (Feldene<sup>R</sup> "A" and Unicom<sup>R</sup> "B") is shown in Fig.2. No statistically significant differences were observed between the two products at any time period over the entire sampling interval.

The mean values of the pharmacokinetic parameters ( $T_{lag}$ ,  $T_{max}$ ,  $C_{max}$ ,  $AUC_{0-24h}$ ,  $AUC_{0-144h}$ ,  $AUC_{0-inf}$ ,  $K_e$  and  $T_{1/2e}$ ) are presented in Table 2. With the exception of the  $T_{max}$  parameter, no statistically significant differences were observed between the two products for the derived pharmacokinetic parameters. The significantly shorter  $T_{max}$  value for Unicom<sup>R</sup> compared to Feldene<sup>R</sup> might be viewed as an advantage for the first product, since it reflects apparently a faster rate of drug absorption which is obviously desirable when single doses are administered for the management of acute conditions.

It was not possible in this investigation to calculate the absorption rate constant and half-life of absorption, because the concentration-time profiles were characterized by multiple peaks, and in the majority of subjects, the profiles were not associated with a clear absorption phase. Concerning the relative extent of absorption, assessed by AUC ratio (B/A) for three time intervals (24 hours, 144 hours, and infinity), the individual and mean values were calculated. The average values with their 95% confidence limits were respectively  $0.98 \pm 0.04$  (0.90-1.06),

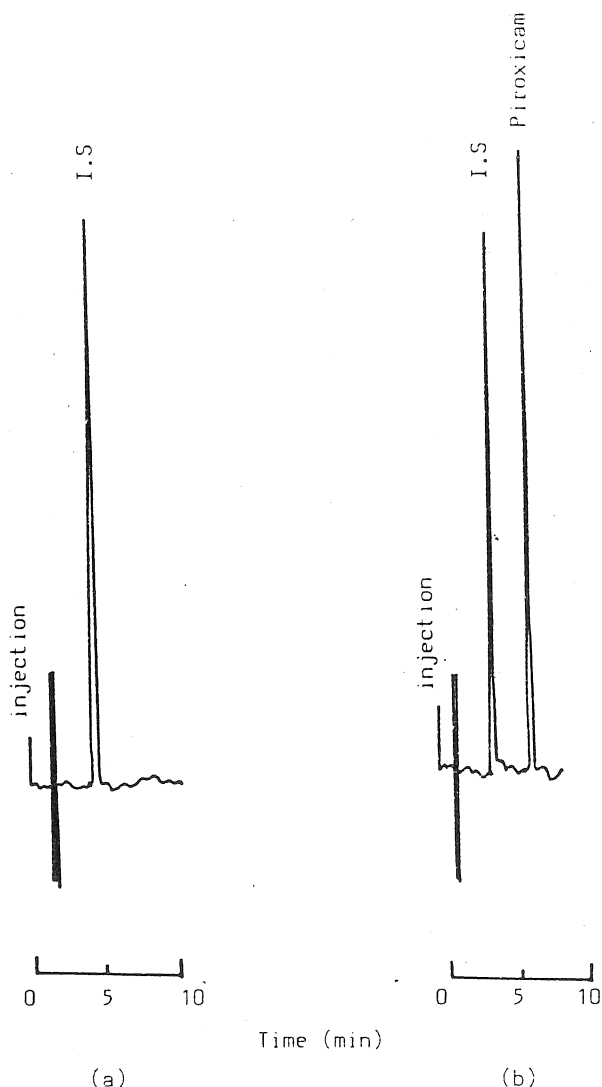


Fig.1.(a) Chromatogram of blank serum spiked with 375 ng of I.S (b) chromatogram of serum sample (955 ng/ml piroxicam) after a single oral dose of 20 mg piroxicam

Table 1. Within-day and between-day precision of piroxicam in plasma

Within-day			Between-day		
Added (µg/ml)	Measured (µg/ml)	Percentage Bias+	Added (µg/ml)	Measured (µg/ml)	Percentage Bias+
0.400 Mean SD CV%	0.412 0.02 4.85	3.00	0.400 Mean SD CV%	0.423 0.015 3.64	5.75
1.5 Mean SD CV%	1.51 0.035 2.32	0.78	1.500 Mean SD CV%	1.523 0.038 2.51	1.53
3.5 Mean SD CV%	3.56 0.18 5.25	1.71	3.500 Mean SD CV%	3.583 0.238 6.63	2.37

\*Mean values represent Six different plasma samples for each conc.  
+Bias = 100 X {(measured conc. -added conc.)/added conc.}

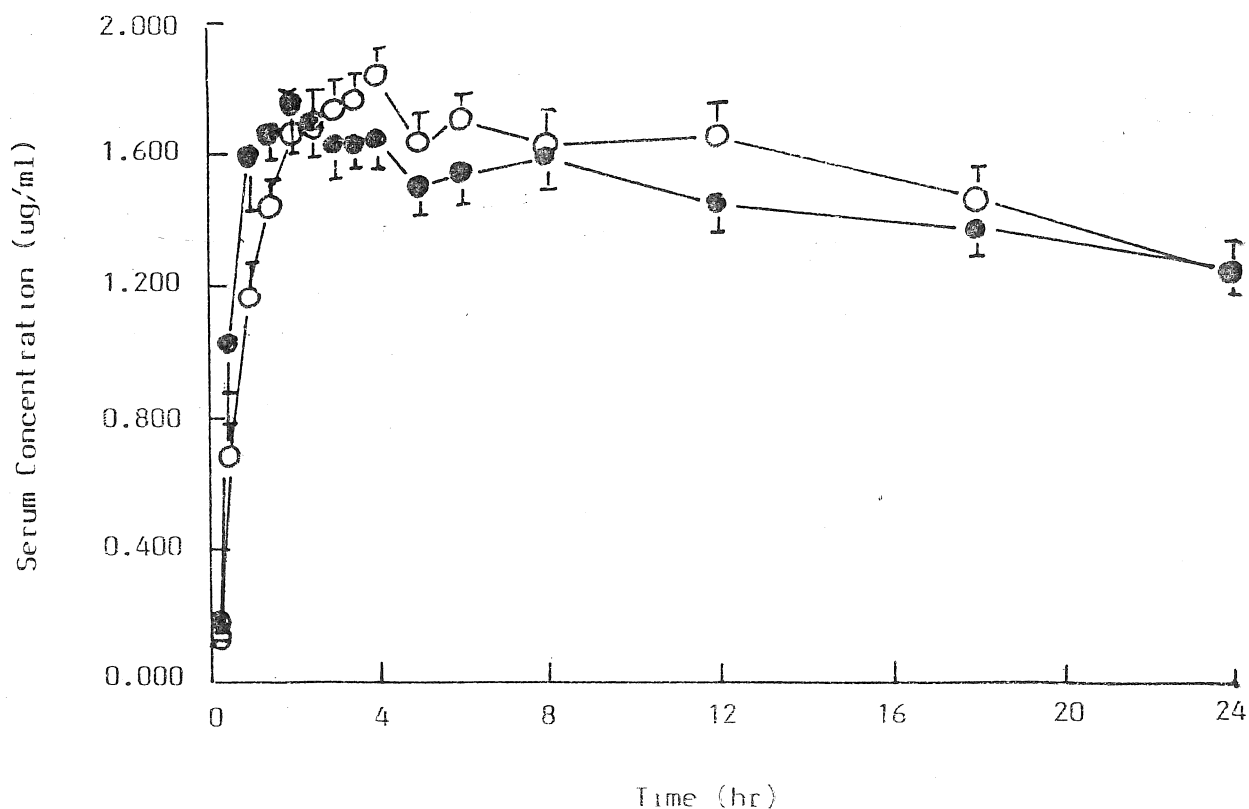


Fig.2. Time versus mean serum concentration of Piroxicam following the administration of a capsule of reference formula (○) and test formula (●)

Table 2. A statistical comparison of the average values ( $\pm$  s.e.m.) of the pharmacokinetic parameters of piroxicam derived from the individual concentration-time curves of Feldene and Unicom following oral administration (1 X 20 mg capsule) to 20 subjects

Pharmacokinetic Parameter	Product		P Value*
	Feldene <sup>R</sup>	Unicom <sup>R</sup>	
T <sub>lag</sub> (h)	0.33 $\pm$ 0.03 (0.27 - 0.39)	0.34 $\pm$ 0.04 (0.26-0.42)	>0.05
T <sub>max</sub> (h)	4.3 $\pm$ 0.6 (3.1 - 5.5)	2.4 $\pm$ 0.4 (1.6 - 3.2)	<0.05
C <sub>max</sub> ( $\mu$ g/ml)	2.07 $\pm$ 0.11 (1.85 - 2.29)	2.03 $\pm$ 0.12 (1.79 - 2.27)	>0.05
AUC <sub>0-24h</sub> ( $\mu$ g.h/ml)	3.6 $\pm$ 2 (32 - 40)	34 $\pm$ 2 (30 -38)	>0.05
AUC <sub>0-144h</sub> ( $\mu$ g.h/ml)	108 $\pm$ 9 (90 - 126)	105 $\pm$ 7 (91 - 119)	>0.05
AUC <sub>0-inf</sub> ( $\mu$ g.h/ml)	124 $\pm$ 12 (100 - 148)	121 $\pm$ 10 (101 - 141)	>0.05
K <sub>e</sub> (h <sup>-1</sup> )	0.016 $\pm$ 0.001 (0.014 - 0.018)	0.017 $\pm$ 0.001 (0.015 - 0.019)	>0.05
T <sub>1/2e</sub> (h)	45 $\pm$ 3 (39 - 51)	46 $\pm$ 4 (38 - 54)	>0.05

Note: Values between brackets represent the 95% confidence limits.

\*: To-way analysis of the variance on log-transformed data was performed for statistical analysis.

1.02  $\pm$  0.06 (0.90-1.14) and 1.02  $\pm$  0.05 (0.92-1.12).

The lack of significant between the two products in either the mean concentration-profiles or in the derived pharmacokinetic parameters (T<sub>lag</sub>, C<sub>max</sub>, AUC<sub>0-24h</sub>, AUC<sub>0-144h</sub>, AUC<sub>0-inf</sub>, T<sub>1/2e</sub>, and K<sub>e</sub>), as well as the finding that the 95% C.L. of the AUC ratios fell within the FDA accepted limits of bioequivalent product (0.8-1.25) clearly indicated that Unicom<sup>R</sup> and Feldene<sup>R</sup> are bioequivalent.

### Acknowledgement

The authors wish to acknowledge the financial support provided by United Pharmaceutical Company., Ltd, Amman, Jordan, and by the Deanship of Scientific Research at Jordan University of Science and Technology.

### References

1. Wiseman, E.H.: Royal Society of Medicine, International Congress and Symposium Series No 1, 11 (1978)
2. Heynen, G.: Royal Society of Medicine, International Congress and Symposium Series No 67, 19 (1984)
3. Abruzzo, J.L., Gordon, G.V., Meyers, A.R.: European Rheumatology and Inflammation 8, 114 (1982)
4. Schattenkirchner, M., Muller-Fassbender, H., Melzer, H., Henze, K.: American Journal of Medicine 72 (2A), 54 (1982)
5. Mihalic, M., Hofiman, H., Kufinec, J., Krile, B., Caplar, V., Kajfez, F., Blazvic, N.: Analytical Profiles of Drug Substances 15, 509 (1986)
6. Twomey, T.M., Hobbs, D.C.: Federation Proceedings 37, 271 (1978)
7. Hobbs, D.C., Twomey, T.M.: J. Clinical Pharmacol. 19, 270 (1979)
8. Fourtillan, J.B., Dubourg, D.: The Rheumatological Disease Process and Symposium Series No 67, 85 (1984)
9. Sheikh Salem, M., Alkaysi, H.N., Gharaibeh, A.M., Gharaibeh, N.M., Badwan, A.: J. Clinical Pharmacy and Therapeutics 19, 203 (1994)