

Bioavailability Studies of Ketorolac Tromethamine Fast Dissolving Tablets Prepared By Direct Compression Method

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Abstract: This study was concerned with the investigation of acute pharmacological responses (pharmacodynamics) including analgesic and anti-inflammatory effects of ketorolac tromethamine fast dissolving tablets prepared by direct compression method using 3% croscarmellose sodium as a superdisintegrant by applying tail flick test and carrageenan induced rat paw edema test respectively.

Also, the work aimed to develop a sensitive LC-MS/MS method for simultaneous determination of ketorolac tromethamine in human plasma samples released from fast dissolving tablets. Also, to estimate whether the prepared tablet dosage form increases the bioavailability of ketorolac tromethamine in the body compared to marketed conventional tablet. From this study, it could be inferred that ketorolac tromethamine fast dissolving tablets (G5) containing (3% croscarmellose sodium as a superdisintegrant, 30% Avicel pH102, 5% aspartame, 1% talc, 1% magnesium stearate, and mannitol Q.S.) and prepared by direct compression method could be considered as a promising formula to enhance bioavailability of the drug.

Keyword: Ketorolac tromethamine fast dissolving tablet , Tail flick, Rat paw edema and bioavailability

I. Introduction

Mechanism of inflammation can be studied by various investigational models of inflammatory process. Also, such models can estimate the anti-inflammatory effectiveness of different active substances⁽¹⁾. Rat paw edema is one of these models in which a suspension of carrageenan is injected into the paw⁽²⁾ or pleural cavity of the rat. The paw volume either measured plethysmographically or after certain interval the animal is killed and the pleural cavity washed. Analgesic effects of anti-inflammatory drugs can be also investigated by several models. The methods used to assess the efficiency of analgesics can manipulate the interpretation of the results of experimental pain studies. Thermal methods include the hot-plate test and the tail flick test. Rodents are placed on a heated surface in the range 50-55°C. Such temperature is as much as necessary to cause distress without tissue damage. Animals licked their forelegs and/or jumped as an indication of pain⁽³⁾. The tail flick test uses radiant heat or immersion in hot water. Mechanical methods involve the application of pressure to a digit, ear or tail and the response is noted.

HPLC coupled to a mass spectrometer (LC-MS) is very important in analysing complex sample matrices where compounds may be present in very low concentrations. Very small molecular size compounds and very large molecular size compounds are easily detected by LC-MS. LC-MS/MS is the most commonly used, due to its sensitivity, ability in detecting polar compounds and its ability to provide structural information⁽⁴⁾. Bioavailability can be defined as the rate and extent of therapeutically active drug absorbed from the drug product, reaching the systemic circulation and become available to the site of action⁽⁵⁻⁶⁾.

II. Material and methods

2.1. Materials

- Ketorolac tromethamine powder was supplied by (El-Amriya Pharmaceutical Ind. Egypt)
- Fam® tablets (10 mg Acapi pharmaceutical co. Egypt), Acetonitril and Ammonium fumarate HPLC grade (Riedel-de Haën, Sigma-Aldrich, and GmbH. Germany)
- Carrageenan sodium (BDH, Poole. England)
- Diethyle ether (Riedel-de Haën, Sigma-Aldrich, GmbH. Germany).

2.2 Animals

Adult male Sprague Dawley albino rats of 150-170 gm body weight, six rats in each group (four groups of rats were used). They were used as a model animal for investigation of analgesic and anti-inflammatory activity of ketorolac tromethamine. Animals were obtained from the animal house of National Center of Research. All animals were maintained under controlled environment at 20 °C with 12 hours dark / light cycle and 50-70% relative humidity. They were fed a standard commercial rodent pellet diet and water.

2.3. Tail flick test⁽⁷⁾:

Each animal was placed gently on the tail flick such that the tail is subjected to the IR beam. Latency to exhibit nociceptive responses, such as removing the tail was determined 30, 60, and 120 min after administration of test substances or saline using Tail flick unit (UGO, Basile, Comerio, Italy) . Saline was administered in one group (group 1) of animals subcutaneously (s.c.) and served as a control. All drugs were injected orally 30 minutes before placing the animal on the hot plate. Treated animals in groups 2 and 3 received ketorolac tromethamine fast dissolving tablets in doses 10mg and 5 mg respectively while treated animals in group 4 recieved commercial oral tablets in a dose of 10 mg

2.4. Carrageenan induced rat paw edema test⁽⁸⁾:

One hour after the administration of the compounds, carrageenan suspension (0.1 ml of 1% w/v suspension in 0.9% saline solution) was injected into the sub planter region of left hind paw of animals. Immediately, the paw volume was measured " initial paw volume" using plethysmometer (Water plethysmometer, apparatus for biological research UGO Basile 21025 Comerio, Italy) Edema volume of control (Vc) and volume of treated (Vt) were used to calculate percentage (%) inhibition and (%) edema volume by using the following formula :

$$\% \text{ Inhibition} = [1 - (Vt/Vc)] \times 100$$

$$\% \text{ Edema volume} = 100 \times \frac{\text{Edema volume after drug treatment}}{\text{Initial volume}}$$

Where Vt and Vc are the increase in the volume of the carrageenan injected paws of treated and non treated control animal groups respectively.

2.5. Statistical analysis:

Values were expressed as means \pm S.E. Comparisons between means were carried out using one way (ANOVA) followed by least significant difference (LSD) and Tukey multiple comparisons test.

(P<0.05) was accepted as being significant in all types of statistical tests. SPSS software (version 17) was used for statistical analysis.

III. Pharmacokinetic study of ketorolac tromethamine fast dissolving tablets.

3.1. Subject population

Six healthy male volunteers (aged between 30-45 years) contributed in this study. Their body weights ranged from 60 to 75 kg. Complete medical history and laboratory analysis for complete hematological and biochemical examination were done to confirm healthy condition of all volunteers. The uptake of any drug was prohibited one week prior to and during the study. Also nicotine utilization was omitted 12 hours before and 24 hours after administration of the drug.

3.2. Study design

The study was executed to compare pharmacokinetics of one ODT (G5) with a standard tablet (Fam®) following administration of (10 mg) each using non-blind, two treatments, two periods, randomized cross-over design. Under the design, half of the subjects were given G5 first treatment and (Fam®) second treatment, and the second half of the subjects were given the treatment in the contradictory order. The subjects received the treatments at 8:00 AM of the day of study after overnight fast (at least 12 hours) as instructed before the study. From this time, they were maintained at the study room under controlled scheme for dietary and liquid intake until the study was completed. No food was allowed for 4 hours after dosing. The wash-out period was one week. The subjects were under medical control during the period of study and were observed for any adverse effects of the drug. The ODT were administrated orally without water, and each subject was asked to maintain it in the mouth for few minutes until completely dissolved in the saliva, then water was allowed after 30 minutes (treatment 1). Fam® tablet was given to the subjects with a full glass of water (about 250 ml) (treatment 2).

3.3. Collection and preparation of blood samples

Venous blood samples were collected in heparinized glass tubes after administration of the dosage form at the following time intervals (0.083, 0.166, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, and 24

hr). All samples were collected and centrifuged at 4000 r.p.m. for 10 minutes to obtain the plasma. The obtained plasma was then subjected to the following steps before injection:

- 1) 0.5 ml plasma + 0.5 ml HCL 1 N + 50 ul (toltrudine 1 ug/ml) + 6 ml Diethylether.
- 2) Vortex, centrifuge, decant upper organic layer.
- 3) Evaporate under a stream of nitrogen at 50 °C.
- 4) Reconstitution in 0.3 ml mobile phase
- 5) Inject 10 ul to LC/MS/MS.

3.4. Analytical procedures for determination of ketorolac tromethamine in plasma

3.4.1. Chromatographic conditions

Plasma concentrations of ketorolac tromethamine were determined using simple procedure using UV detector at wave length (λ_{\max}) 312 μm .

Mobile phase: 10 mM amm. formate : Acetonitrile (30:70 v/v) .
Flow rate: 0.7 ml/min.
Column : inertsil ODS -3 (5 μm x 4.6 mm x 50 mm)
Injection volume: 10 μl .

3.4.2. Method for validation of bioavailability

a) Selectivity

Plasma samples (six blanks) were chromatographed before use in order to test out for endogenous constituents, which might impede with ketorolac tromethamine. Spiked plasma samples representing a low, medium and high ketorolac tromethamine (QCL, QCM, and QCH, respectively) were also analysed to verify the selectivity of the analysis method.

b) Linearity and linear working range

The linearity and linear working range were determined from the constructed standard calibration curve.

3.4.3 .Preparation of stock and working standard solutions

Ketorolac tromethamine standard stock solution was prepared by dissolving 10 mg of the standard drug into 100 ml methanol.

3.4.4. Construction of in-vivo standard calibration curve

ketorolac tromethamine stock solution was diluted with methanol to contain (10 mg) of the drug, by transferring 1 ml of stock solution into centrifuge tubes provided with tight sealing polyethylene cap

Standard solutions were prepared by spiking 0.5 ml of blank plasma over 1ml of each of the above standard solutions. The analytical method consisted of a single step extraction of ketorolac from plasma using a mixture of (0.5 ml HCL 1 N), 50 ul (toltrudine 1 ug/ml), and 6 ml Diethylether.

The samples were then vortexed using Vortex mixer (Paramix II, Julabo laboratechnik, GmbH, Seelbak, Germany) for 5 minutes and centrifuged at 4000 r.p.m. for 10 minutes. The upper layer was collected and transferred to another tube, filtered through 0.45 μm Millipore filter, then evaporated under a stream of nitrogen at 50 °C. This was followed by reconstitution in 0.3 ml mobile phase. Finally, 10 μl were injected to the column for analysis.

3.4.5. Plasma analysis

Each blood sample was prepared for chromatographic assay by adding a mixture of (0.5 ml HCL 1 N), 50 ul (toltrudine 1 ug/ml), and 6 ml Diethylether. The samples were then vortexed for 5 minutes and centrifuged at 4000 r.p.m. for 10 minutes. The upper layer was collected and transferred to another tube, filtered through 0.45 μm Millipore filter, then evaporated under a stream of nitrogen at 50 °C. This was followed by reconstitution in 0.3 ml mobile phase. Finally, 10 μl were injected to the column of analysis.

3.4.6 Assessment of pharmacokinetic parameters⁽⁹⁾

For single –dose studies, pharmacokinetic data include evaluation of the plasma concentration time data for each subject, $C_{p_{\max}}$ ($\mu\text{g}/\text{ml}$), T_{\max} (hours), $AUC_{(0-24)}$ ($\mu\text{g. hr}/\text{ml}$), $AUC_{(0-\infty)}$ ($\mu\text{g. hr}/\text{ml}$), K (hour^{-1}), $t_{1/2}$ (hours), MRT, and F_{rel} were estimated using a computer program Win- Non- Lin (version 1.5, Scientific consulting, Inc, NC).

IV. Results and discussion

Rats treated with either the selected ketorolac tromethamine ODT formula (G5) or Fam® tablets prolonged the tail flick reaction time of the animals and demonstrated significant antinociceptive action ($P < 0.05$: statistically significant from the control) as shown in table (1). There was no significant differences between the prepared KT fast dissolving tablets and Fam® tablets ($P > 0.05 = 0.263$). Both tablets showed good analgesic activity but the prolonged reaction time was observed with ketorolac tromethamine ODT tablets in a dose of 10 mg.

It was apparent that injection of carrageenan induced prominent edema in all the tested animals. Moreover, there was a significant difference ($p < 0.05$) between all groups receiving KT tablets and the control group as represented in tables (2& 3). The anti-inflammatory activity of the selected ketorolac tromethamine ODT formula (G5) either in a dose of 10 mg or 5 mg appeared clearly from the first hour and remained till the fourth hour, while the anti-inflammatory activity of the commercial tablet Fam® was almost existed during the first hour as shown in figure (1). The results inferred that the prepared KT fast dissolving tablets gave a higher percentage of edema inhibition than the oral conventional tablets (61% and 53% respectively).

**Results of the validation of the analytical method revealed that

- The method was selective for ketorolac tromethamine with a minimum limit of quantification (10 ng/ml) as in figure (2& 3).
- Excellent linearity of the calibration lines and high reproducibility of the assay method was obtained as in figure (4).
- The tested market tablets Fam® exhibited a mean peak plasma concentration of 934.185 ± 0.707 ng/ml as illustrated graphically in figure (5), a mean T_{max} of 2.5 hours, a mean $AUC_{(0-24)}$ of 7996.71 ± 20.046 ng.hr/ml, and a mean $AUC_{(0-\infty)}$ of 8382.82 ± 192.781 ng.hr/ml as represented in table (4).
- The selected ODT formula (G5) showed a mean peak plasma concentration of 1220.73 ± 0.804 ng/ml as represented graphically in figure (6 & 7), a mean T_{max} of 1 hours, a mean $AUC_{(0-24)}$ of 10178.29 ± 9.743 ng.hr/ml, and a mean $AUC_{(0-\infty)}$ of 10180.804 ± 9.743 ng.hr/ml as shown in table (5).
- The relative bioavailability was found to be 127.302 ± 0.385 based on the mean of $AUC_{(0-24)}$ of the tested formula G5 compared to that of the reference standard product.
- The statistical analysis of $c_{p,max}$, $AUC_{(0-24)}$, and $AUC_{(0-\infty)}$ inferred that a significant difference ($p < 0.05$) in the rate of drug absorption was found between the selected KT fast dissolving tablet formula G5 and the commercial conventional tablet Fam®.

From all the previous findings, it could be inferred that ketorolac tromethamine fast dissolving tablets (G5) containing (3% croscarmellose sodium as a superdisintegrant, 30% Avicel pH102, 5% aspartame, 1% talc, 1% magnesium stearate, and mannitol Q.S.) and prepared by direct compression method could be considered as a promising formula to enhance bioavailability of the drug.

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References

- [1]. Romay C, Ledón N, and González R. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflam.Res.*1998: 334-338.
- [2]. Kumar KEE. Synthesis and anti-inflammatory activity of N-substituted dihydropyridylacetic acids, esters and amides. *Drug Design Discov.*1978 : 145-149.
- [3]. Young-Liang JIA, Zhao J, Zhang LH, et al. Analgesic and anti-inflammatory effects of ginger oil. *Chines Herbal Medicines.* 2011: 15-155.
- [4]. Bajad S, and Shulaev V. Highly parallel metabolomic approaches using LC-MS/MS for pharmaceutical and environmental analysis. *Trend in Analytical Chemistry.*2007: 625-636.
- [5]. Gibaldi M. *Biopharmaceutics and clinical pharmacokinetics.* 3rd ed. Lea & Febiger. Philadelphia. 1984; pp131-136.
- [6]. Schoenwald RD. *Introduction in pharmacokinetics in drug discovery and development.*3rd ed. CRC Press. London. (2002): pp 3-30.
- [7]. D'Amour FE, and Smith DL. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.*1941: 74- 79.
- [8]. Winter CA, Risely E.A, and Nuss GW. Carrageenan induced edema hind paw of the rats as an assay for anti inflammatory drugs. *Proc. Soc. Expt. Bio. Med.*1962: 544-547.
- [9]. Webster AA. *Pharmaceutical product stability.* In: *Pharmaceutical sciences Encyclopedia: Drug discovery, development and manufacturing* (Webster AA).Jon Wiley and Sons, Inc.New York. 2010;pp 1-14.

Table (1): Tail flick reaction time of the selected ketorolac tromethamine fast dissolving tablets (G5) and commercial conventional tablet Fam®

Group No	Tail flick reaction time after the following time intervals in minutes (Mean ± SE)			
	Baseline	30	60	120
Group 1	3.64±0.743	4.34±0.344	4.74±0.350	5.82±0.481
Group 2	5.22±0.333	7.44±0.655*	10.16±0.595*	12.08±0.690*
Group 3	3.74±0.746	5.80±0.659	8.20±1.126*	11.34±1.763
Group 4	2.28±0.1393	4.52±0.539	6.22±0.879	7.54±0.643

Table (2): Anti-inflammatory activity of the selected ketorolac tromethamine fast dissolving tablets (G5) and the conventional commercial tablets (Fam®)

Group no	% edema after the following time intervals in minutes (Mean ± SE)			
	60	120	180	240
Group 1	100 ± 15	129 ± 8	143 ± 16	131 ± 19
Group 2	69 ± 14	58 ± 7*	55 ± 9*	65 ± 8*
Group 3	79 ± 7	77 ± 11*	58 ± 7*	66 ± 8*
Group 4	90 ± 15	78 ± 19	67 ± 14*	79 ± 17

• Values are expressed as means ± SE (n = 6).

* Significantly different from control group at P < 0.05.

Significantly different from reference drug group at P < 0.05.

Statistical analysis was done using one way ANOVA followed by Turkey for multiple comparisons respectively.

Table (3): (%) Inhibition of rat paw edema for the selected ketorolac tromethamine fast dissolving tablets (G5) and the conventional commercial tablets Fam®

Group no	% Inhibition in edema volume after the following time intervals in minutes			
	60	120	180	240
Group 1	-	-	-	-
Group 2	31	55	61	50
Group 3	21	40	57	49
Group 4	10	40	53	69.39

Table (4): Pharmacokinetic parameters of ketorolac tromethamine following administration of Fam® 10 mg to six healthy volunteers

Parameter	A	B	C	D	E	F	Mean ± SD
C _{pmax} (ng/ml)	934.59	934.96	934.57	933.95	934.09	932.95	934.19±0.707
T _{max} (hr)	2.5	2.5	2.5	2.5	2.5	2.5	2.5 ± 0.00
AUC ₍₀₋₂₄₎ (ng.hr/ml)	7999.02	8009.67	7968.49	8023.18	7978.95	8000.64	7996.71 ± 20.046
AUC _(0-∞) (ng.hr/ml)	8001.46	8499.77	8391.38	8523.54	8430.19	8450.56	8382.82 ± 192.781
T _{1/2} (hr)	5.629	5.709	5.506	5.735	5.880	5.589	5.626±0.085
K (hr ⁻¹)	0.123	0.121	0.126	0.121	0.124	0.124	0.123±0.002
MRT	7.484	8.452	8.271	8.474	8.340	8.348	8.228±0.372

Table (5): Pharmacokinetic parameters of ketorolac tromethamine following administration of an oral dose of 10 mg of the selected ODT (G5) to six healthy volunteers

Parameter	A	B	C	D	E	F	Mean ± SD
C _{pmax} (ng/ml)	1220.48	1219.96	1221.98	1220.04	1221.42	1220.48	1220.73±0.804
T _{max} (hr)	1	1	1	1	1	1	1 ± 0.00
AUC ₍₀₋₂₄₎ (ng.hr/ml)	10168.50	10172.96	10175.92	10186.53	10193.73	10172.09	10178.29 ± 9.743
AUC _(0-∞) (ng.hr/ml)	10171.00	10175.49	10178.43	10189.05	10196.24	10174.61	10180.80 ± 9.743
T _{1/2} (hr)	5.791	5.847	5.802	5.817	5.809	5.820	5.814±0.019
K (hr ⁻¹)	0.119	0.1185	0.119	0.1191	0.1192	0.1190	0.1191±0.0004
MRT	7.219	7.235	7.221	7.233	7.235	7.223	7.228±0.007
% Relative bioavailability	127.122	127.003	127.762	126.964	127.757	127.204	127.302±0.365

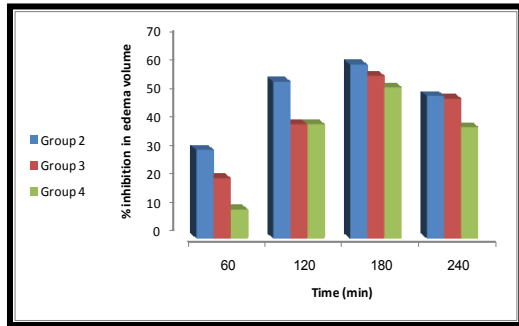
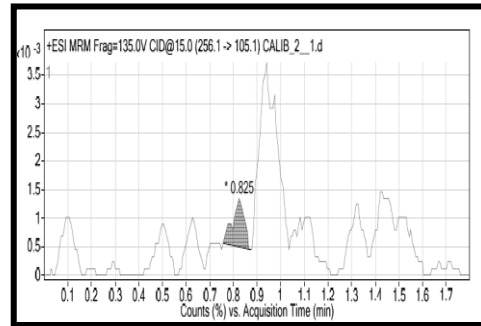


Fig (1): Anti-inflammatory efficacy of the selected ketorolac tromethamine fast dissolving tablet (G5) and the conventional commercial tablet Fam®.



Fig(2): A typical chromatogram obtained from a processed blank human plasma

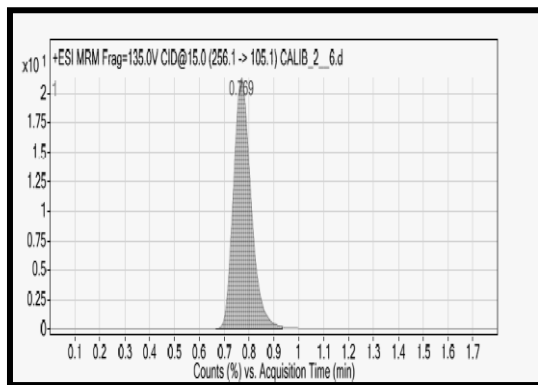


Fig (3): Medium Quality Control sample of ketorolac tromethamine tromethamine (QCM)

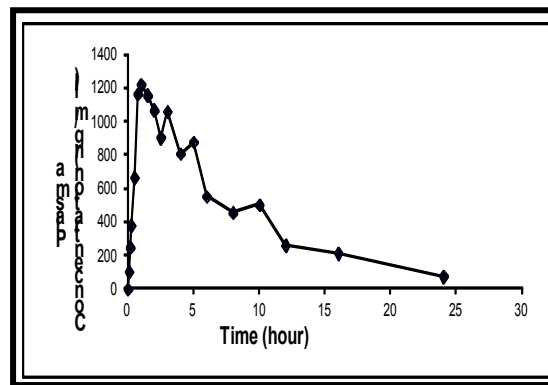


Fig (6): Plasma concentration of ketorolac following administration of an oral dose of 10 mg of the selected ODT formula (G5) in six healthy volunteers

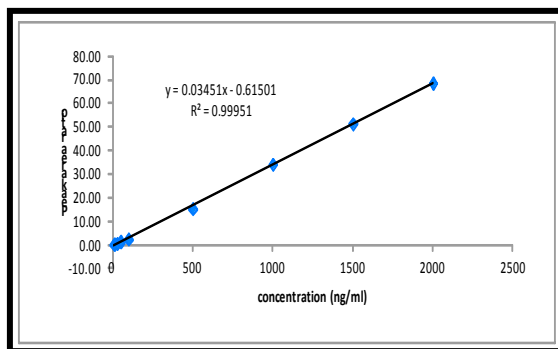
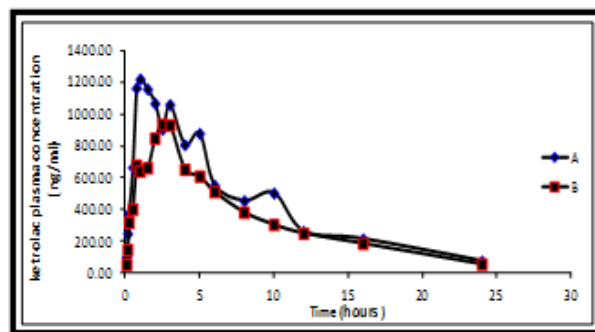


Fig (4): In-vivo standard calibration curve of ketorolac tromethamine in human plasma



Fig(7): Mean plasma concentrations of ketorolac

tromethamine after administration of an oral dose of 10 mg of Fam® and the selected fast dissolving tablet Formula G5 in six healthy volunteers

- A : Selected KT fast dissolving tablet formula G5
- B : Commercial conventional KT oral tablet Fam®

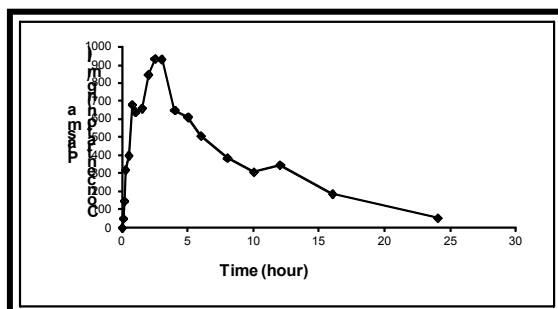


Fig (5): Plasma concentration of ketorolac tromethamine following the administration of an oral dose of Fam® tablets 10 mg to six healthy volunteers.