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RESEARCH PAPER

Comparative Pharmacokinetics of New Theophylline Preparations as Egifilin® 200 mg and 400 mg Retard Tablets After Single Dose in Healthy Volunteers

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ABSTRACT

Pharmacokinetic properties of Egifilin® retard tablets (containing 200 or 400 mg of theophylline, EGIS Pharmaceuticals Ltd., Budapest, Hungary) were investigated in 12 healthy volunteers (six women, six men). Pharmacokinetic analysis was performed according to a one-compartment open model. The retard nature of both 200 and 400 mg tablets could be demonstrated. Comparison of the pharmacokinetic curves of women and men indicated that there was no sex difference in the pharmacokinetics of two retard tablets.

Egifilin retard tablets ensured plasma levels for almost 30 hr after single drug intake with an extremely long retard plateau between 6 and 30 hr. For study purposes, an improved rapid HPLC-UV analytical method (less than 3.5 min chromatogram) has been elaborated for the determination of theophylline in human plasma. The range of the calibration is 0.6–18 μg/ml.

INTRODUCTION

Theophylline (1,3-dimethylxantine), the alkaloid compound, can be regarded as a classical medicine. Its main indications are relief and/or prevention of symptoms from bronchial asthma and reversible bronchospasm associated with chronic bronchitis and emphysema.

Considering the high number of patients requiring chronic theophylline treatment, adequate retard theophyl-

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line preparations seem mandatory; they help in finding the appropriate dose quickly and the retard effect prevents the occurrence of high peak plasma concentrations, thereby decreasing the incidence and severity of side effects. Because they ensure therapeutic plasma levels continuously, retard preparations (perhaps with once-a-day administration) may prevent the appearance of asthmatic symptoms.

Upon oral administration, theophylline is absorbed rapidly and completely. Maximum plasma concentration (C_{max}) develops within 2 hr. The biological half-life of theophylline is 3.5 hr in children and 8–9 hr in adults. The therapeutic plasma level ranges between 8 and 16 (10–20) μg/ml in adults and between 7 and 14 μg/ml in children (1,2,3,4), although in some cases bronchospasms could be eliminated with 5 μg/ml plasma concentration (1). Above 20 μg/ml plasma level toxic symptoms appear: nausea, vomiting, tachycardia, and in severe cases, spasm and extrasystole.

Because of its 8–9-hr-long elimination half-life, the plasma level of theophylline decreases significantly by dawn; therefore, sustained-release preparations providing high enough plasma levels to prevent bronchospasm also in the critical time can successfully be used to avoid early morning distress.

The pharmacokinetic properties and retard effects of the new Egifilin 200 and 400 mg tablets were investigated in 12 healthy volunteers in a single dose, single blind, crossover study. All the important pharmacokinetic parameters ($AUC_{0-\infty}$, AUC_{0-48}, C_{max}, HVD, MRT, t_{max}, k_a, β, $t^{\beta}_{1/2}$, V_d, Cl) were calculated. For the determination of theophylline, a new improved rapid reversed-phase HPLC-UV method has been elaborated.

SUBJECTS AND METHODS

Protocol for Clinical Investigations

Single dose comparative pharmacokinetic studies were carried out on both retard theophylline preparations (200, 400 mg) on the basis of study protocols approved by the study directors, ethical committee of the study site, and the National Institute of Pharmacy. Healthy volunteers participating in the study were selected and the studies were conducted according to Hungarian and internationally accepted guidelines and recommendations and in the spirit of the revised Helsinki Declaration (Hong Kong, 1989) (5). The study had a single dose, single blind, two period, crossover design. The 12 volunteers (6 women, 6 men) between the age of 20 and 50 years (Table 1) assessed as healthy based on medical history, physical, and routine laboratory examinations were admitted to the study treatment. All subjects gave written informed consent before entering the study. The intake was carried out in a random way; either a 200 mg or 400 mg tablet was given and a 6-day wash out period was allowed before the next treatment. In both treatment periods blood samples were

Table 1

Data of Healthy Volunteers Participating in the Pharmacokinetic Study of Egifilin

Volunteer Serial No.	Code	Sex	Age (years)	Body Weight (kg)	Height (cm)	Body Mass Index (kg/m²)	Randomization Code
1.	K.Cs.	M	29	80	176	25.8	B/A
2.	K.Sz.E.	W	28	62	168	21.9	A/B
3.	C.M.	W	22	63	170	21.8	A/B
4.	N.B.	M	49	79	180	24.4	B/A
5.	L.Cs.	W	20	70	167	25.1	A/B
6.	B.L.	M	32	60	157	24.3	B/A
7.	T.M.	W	25	65	172	21.9	B/A
8.	Sz.A.	M	25	75	183	22.4	A/B
9.	T.R.	M	33	90	184	26.6	B/A
10.	J.J.	W	20	61	165	22.4	A/B
11.	L.T.	M	44	78	175	25.5	B/A
12.	M.L.	W	40	62	160	24.2	A/B
	Mean		30.5	70.4	171.4	23.8	
	±SD		9.1	9.3	8.2	1.6	

Randomization code: A: 200 mg tablet, B: 400 mg tablet.
Sex code: W: woman, M: man.

collected before treatment (zero time sample) and 1, 2, 3, 4, 5, 6, 10, 12, 16, 24, 30, 36, and 48 hr after treatment. Blood samples were collected into glass centrifuge tubes previously rinsed with 2% heparin solution. Right after they were taken, the blood samples were centrifuged at 3000 rpm for 10 min and the sequestrated plasma was deep-frozen to -20°C until sample preparation. At this temperature, plasma samples could be stored for up to 12 weeks from the time of sample collection.

Test Preparation

Theophylline was purchased from Knoll AG (Ludwigshafen, Germany). Egifilin retard tablets were produced according a patented method of EGIS Pharmaceuticals Ltd. (Budapest, Hungary) (6), and they contain hydrophilic polymer matrix that consists of vinylpyrrolydon-vynilacetate copolymer (produced by BASF, Köln, Germany) and crosslinked acrylic acid polymer (produced by Goodrich, Cleveland, OH). The compositions of the theophylline retard tablets of 200 mg and 400 mg were chosed such that the in vitro dissolution rate of the theophylline from the tablets was essentially the same.

Bioanalytical Method

For the determination of theophylline in human plasma, we improved a previously published HPLC-UV method (7). The changes resulted in more favorable analysis time (less than half that of the original method) and more efficient separation (improved sensitivity and resolution) that led to feasibility for clinical pharmacokinetic studies.

Materials

Theophylline, β-hidroxyethyl-theophylline (as internal standard), and trichloro-acetic acid were purchased from Sigma (St. Louis, MO). Acetonitrile (ACN), acetic acid, ethanol, and HPLC-grade water were manufactured by Fluka Chemie AG. (Buchs, Switzerland). Sodium acetate was purchased from Merck (Darmstadt, Germany). The solvents used were of chromatography grade, the other chemicals were of analytical purity.

Instruments and Chromatographic Parameters

For chromatographic analysis, the HP 1090 M Series II Liquid Chromatograph (Hewlett-Packard, Palo Alto, CA) with autosampler, terner gradient pump, and diode array detector (DAD) were used under control of HP ChemStation Pascal Series software. The separation of the interesting compounds was achieved on Supersphere 100 RP-18, 4 μm, 125 mm × 4 mm analytical column using LiChrosphere 100 RP-18, 5 μm, 4 mm × 4 mm guard column (Merck, Darmstadt, Germany). The separation was performed at 30°C. The flow rate was 1.0 ml/min. The UV (DAD) monitoring was done at 280 nm (4 nm bandwidth) and the reference wavelength was 350 nm (80 nm bandwidth). The mobile phase consisted of 11% ACN and 89% of 0.01 M sodium acetate buffer; pH was adjusted to 4.5 with acetic acid. The solvents were bubbled with high purity helium.

Solutions

Solutions of 1 mg/ml concentration were prepared from the theophylline and the internal standard by dissolving the substances in ethanol with slight heating and diluting the solutions obtained with the eluent. Further dilutions of stock solution of theophylline were prepared with eluent (30, 75, 150, 300, 600, and 900 μl/ml) and 10 μl was added to 0.5 ml plasma in each case. A 1 mg/ml solution of the internal standard was diluted with eluent to obtain the concentration of 600 μg/ml. Ten microliters of this solution was added to 0.5 ml plasma sample (calibrator or clinical plasma samples) before processing.

Sample Processing

To 0.5 ml of plasma samples, 250 μl of 20% trichloro acetic acid was added. The samples were then vortexed (30 sec) and centrifuged at 1000 rpm at 4°C for 10 min. The supernatant was recentrifuged at the same speed and temperature for 5 min. Twenty microliters of the supernatant obtained was injected directly onto the analytical column.

Method Validation

The HPLC method was validated according to internationally accepted criteria (8,9).

Linearity Test

Linearity of the method was tested in the expected concentration range of 0.6–18 μg/ml with 12 parallel determinations. The following calibration points were prepared: 0.6, 1.5, 3.0, 6.0, 12.0, and 18.0 μl/ml.

Precision and Accuracy

Intra-day precision and accuracy were determined at three concentration levels (0.6, 6.0, and 12 μg/ml) by triplicate analyses. For establishing inter-day precision and accuracy, six independent calibrations were measured.

System Suitability Test

The suitability of the chromatographic system was determined by five parallel injections from two spiked plasma samples prepared at two concentration levels (1.5 and 12 μg/ml).

Stability Test

The stability of theophylline (at 6 μg/ml concentration level) in human plasma was tested under storage at -20°C for 12 weeks with one determination each week.

Pharmacokinetic Analysis

The individual pharmacokinetic parameters (AUC_{0-48}, $AUC_{0-\infty}$, AUC_{Rest}, C_{max}, t_{max}, HVD, MRT, k_a, β, $t^{\beta}_{1/2}$, V_d, Cl) were determined on the basis of the plasma concentration versus time data with a one-compartment open model fitted by the peeling technique (10,11) using Siphar/Win ver. 1.12 (Simed SA, Créteil Cedex, France) pharmacokinetic software package.

Half-value duration (HVD), an important parameter of retard preparations, was determined graphically from the polygonal curve fitted to the concentration values. HVD is equivalent to the interval excised by the polygonal curve from the straight line drawn parallel to the time axis at $C_{max}/2$ value (10). Mean residence time (MRT) of active substance in the systemic circulation, the other important parameter of retard preparations, was calculated from the fitted pharmacokinetic curve (12) as $MRT = {}_0\int^{\infty} tC(t)dt / {}_0\int^{\infty} C(t)dt$.

The elimination hybrid rate constant, β, was obtained as the slope of the straight line fitted by the least squares method. The corresponding elimination half-life, $t^{\beta}_{1/2}$, was calculated according to the following formula: $t^{\beta}_{1/2} = \ln 2/\beta$.

In addition to the above parameters, the of value of distribution volume (V_d) and total body clearance (Cl) were calculated according to the following equations: $V_d = Cl/\beta$ and $Cl = Dose/AUC_{0-\infty}$. The results of pharmacokinetic calculations are presented as mean ± SD and SE.

RESULTS AND DISCUSSION

Bioanalysis

The analytical method calibration demonstrated acceptable linearity ($r = 0.9972$, $y = -0.0011592 + 0.084899$; $n = 12$) in the range of 0.6–18 μg/ml. No endogenous peak that would interfere with the determination of theophylline or internal standard was detected

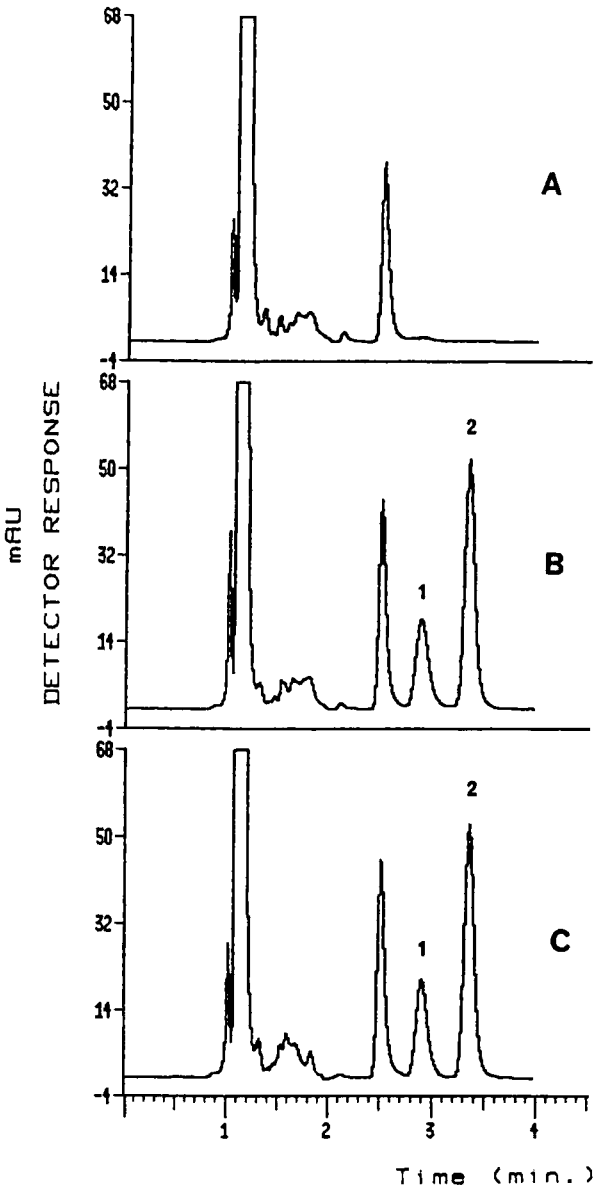

Figure 1. HPLC chromatograms of volunteer no. 1 (A) before, (B) 8 hr, and (C) 24 hr after ingestion of single Egifilin retard 400 mg tablet (1: theophylline, 2: internal standard).

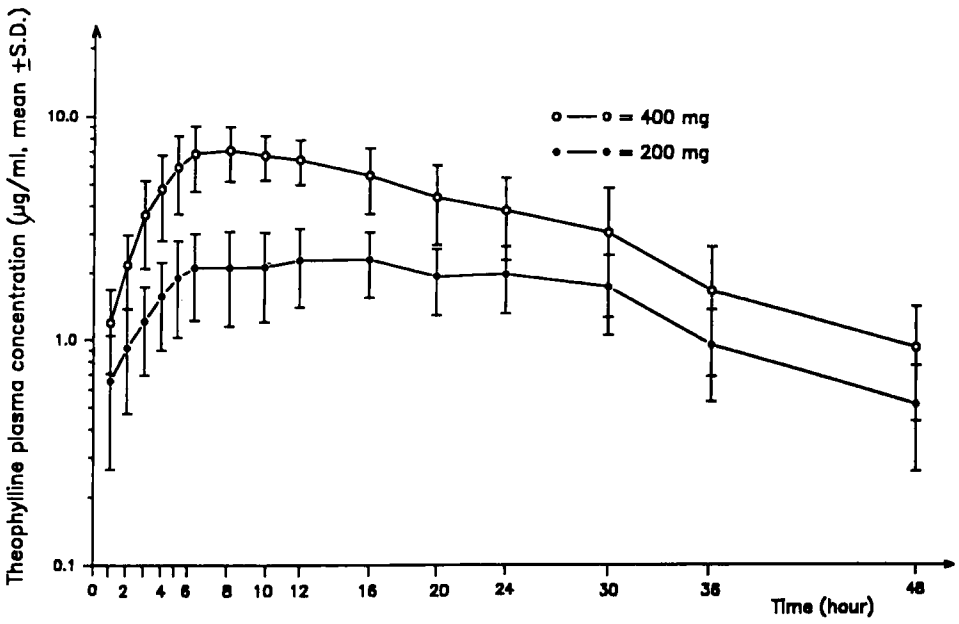

Figure 2. Pharmacokinetics of retard Egifilin tablets.

in the chromatogram of blank plasma (Fig. 1). The resolution between the chromatographic peak of theophylline and that of internal standard amounted to R_S = 1.55. The retention time of theophylline was 2.8 min, while the internal standard was eluted at 3.3 min (Fig. 1).

Intra- and inter-day precision and accuracy values were calculated to determine the reproducibility. The intra-day precision and accuracy values varied between -0.57 and 1.73 RSD% and -2.54 and 8.20%, respectively. The same parameters of inter-day reproducibility fell within the range of 2.65–5.81 RSD% and -1.17–

Table 2

Pharmacokinetic Parameters of 200 mg Retard Egifilin Tablets

Code	AUC_{0-48} (μg hr/ml)	$AUC_{0-\infty}$ (μg hr/ml)	C_{max} (μg/ml)	t_{max} (hr)	β (1/hr)	$t^{\beta}_{1/2}$ (hr)	HVD (hr)	MRT (hr)	V_d (liter)	Cl (liter/hr)
K.CS.	59.36	66.68	1.82	20.00	0.06	11.80	42.57	25.68	51.08	3.00
K.Sz.E.	62.96	67.33	3.16	8.00	0.06	11.22	14.00	20.74	48.06	2.97
C.M.	62.70	68.49	2.61	16.00	0.06	11.15	28.57	24.18	46.98	2.92
N.B.	62.70	66.16	2.37	16.00	0.08	9.22	23.14	21.32	40.21	3.02
L.CS.	52.53	59.91	1.82	12.00	0.05	12.78	24.29	25.66	61.56	3.34
B.L.	68.77	80.26	3.18	12.00	0.07	9.27	31.14	21.94	33.32	2.49
T.M.	55.96	59.47	2.07	5.00	0.08	9.01	29.14	21.47	43.73	3.36
Sz.A.	70.76	88.62	2.66	16.00	0.04	16.29	27.14	32.92	53.04	2.26
T.R.	38.43	46.50	2.02	24.00	0.08	8.74	14.19	21.86	54.23	4.30
J.J.	114.24	125.88	3.83	24.00	0.07	10.22	32.00	25.09	23.42	1.59
L.T.	89.39	101.22	3.22	8.00	0.05	13.67	24.57	26.24	38.96	1.98
M.L.	100.59	129.90	4.37	6.00	0.03	20.94	26.86	32.43	46.51	1.54
Mean	69.86	80.04	2.76	13.91	0.06	12.07	26.47	24.96	45.09	2.73
±SD	21.40	26.51	0.81	6.55	0.01	3.58	7.68	4.09	10.18	0.80
±SE	6.18	7.65	0.23	1.89	0.00	1.03	2.22	1.18	2.94	0.23

Table 3

Pharmacokinetic Parameters of 400 mg Retard Egifilin Tablets

Code	AUC_{0-48} (μg hr/ml)	$AUC_{0-\infty}$ (μg hr/ml)	C_{max} (μg/ml)	t_{max} (hr)	β (1/hr)	$t^{\beta}_{1/2}$ (hr)	HVD (hr)	MRT (hr)	V_d (liter)	Cl (liter/hr)
K.CS.	181.55	202.04	5.59	10.00	0.06	11.65	34.29	25.47	33.26	1.98
K.Sz.E.	198.86	205.70	10.97	5.00	0.08	9.12	14.57	16.03	25.60	1.94
C.M.	165.62	178.86	6.85	8.00	0.06	10.93	15.14	20.95	35.25	2.24
N.B.	117.02	119.01	6.17	8.00	0.10	6.91	23.43	15.38	33.51	3.36
L.CS.	94.54	102.93	4.96	8.00	0.09	7.87	19.71	17.03	44.11	3.89
B.L.	170.07	193.00	6.49	12.00	0.05	15.14	15.71	26.60	45.28	2.07
T.M.	173.89	179.45	10.61	6.00	0.13	5.35	14.86	13.21	17.20	2.23
Sz.A	150.13	156.27	7.26	10.00	0.08	8.35	21.71	17.75	30.83	2.56
T.R.	91.76	96.56	5.43	10.00	0.14	5.03	15.43	12.97	30.07	4.14
J.J.	260.18	279.89	10.58	8.00	0.06	11.02	23.43	20.85	22.72	1.43
L.T.	220.20	257.23	7.58	8.00	0.05	14.03	31.43	28.01	31.47	1.55
M.L.	175.87	189.17	7.08	10.00	0.06	10.72	24.57	21.73	32.70	2.11
Mean	166.64	180.01	7.42	8.58	0.08	9.68	21.18	19.67	31.83	2.46
±SD	49.15	56.07	2.11	1.93	0.03	3.17	6.64	5.11	7.91	0.88
±SE	14.19	16.17	0.61	0.56	0.01	0.91	1.92	1.48	2.28	0.25

8.83%, respectively. For the system suitability test, 1.33 and 1.52 RSD% values were obtained at 1.5 and 12 μg/ml theophylline concentrations, respectively. Spiked plasma samples were stable stored at -20°C for 12 weeks (7).

Pharmacokinetics

During the comparative pharmacokinetic study of two retard theophylline preparations, transient, mild side effects were observed only in two subjects. There was

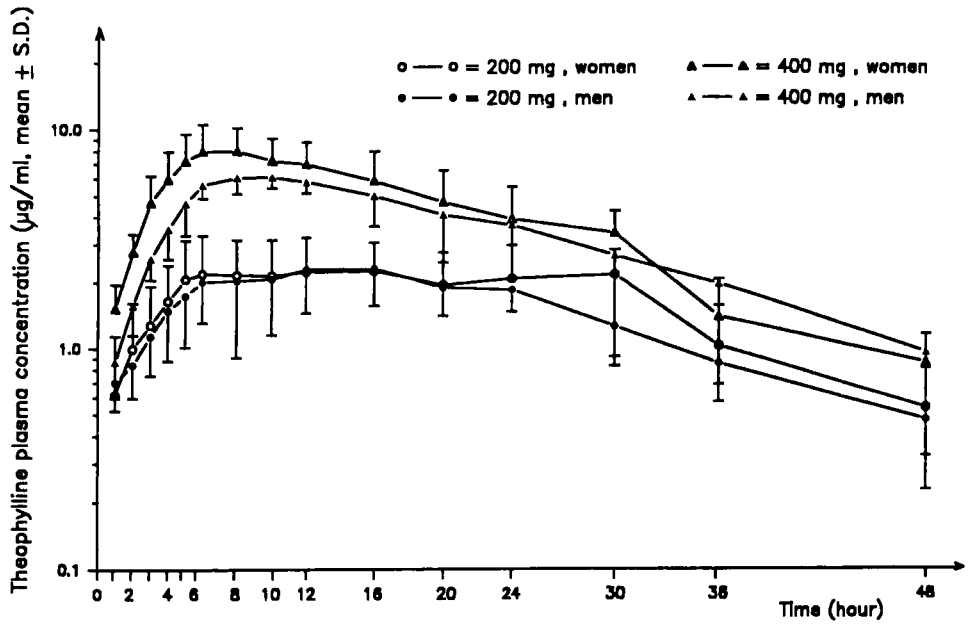

Figure 3. Comparison of pharmacokinetics of retard Egifilin tablets in women and men.

reported tremor, palpitation, and polyuria at volunteer no. 1 after ingesting the 400 mg tablet, and polyuria in volunteer no. 4 after the administration of both the 200 and 400 mg tablet. None of the two preparations had any significant effect on the clinical laboratory parameters tested during the study.

Mean pharmacokinetic curves for 200 and 400 mg tablets are shown in Fig. 2 in semilogarithmic representation. An extremely long retard plateau can be observed between 6 and 30 hr. Individual and mean pharmacokinetic parameter values can be found in Table 2 for the 200 mg and in Table 3 for the 400 mg tablet.

The C_{max} value (2.76 μg/ml) measured after the administration of a single 200 mg retard tablet and the half-value duration (HVD = 26.48 hr) was practically the same as that of the most modern preparations (24 hr). After administration of the 400 mg tablet, the C_{max} value amounted to 7.42 μg/ml and an HVD of 21.18 hr was obtained. The comparison of HVD values of two dose levels was not statistically different at 95% significance level.

The t_{max} values were 13.9 and 8.6 hr in the case of 200 and 400 mg tablets, respectively. The MRT values were 24.9 and 19.6 hr after the administration of 200 and 400 mg tablets, respectively. Comparing the $AUC_{0-\infty}$ values obtained in men and women after treatment with 200 and 400 mg tablets, there was no sex difference found in pharmacokinetics of retard theophylline at either dose level (Fig. 3).

The AUC_{Rest} values of 200 and 400 retard preparations were calculated to 12.72 and 7.43%, respectively.

On the basis of the HVD and MRT values, which are characteristic parameters in retard nature of preparations, the retard character of Egifilin 200 and 400 mg tablets seems to be verified.

Discussion

The retard effect the two newly developed theophylline preparations (Egifilin 200 and 400 mg retard tablets) is ensured for a period of about 24 hr with a long retard plateau between 6 and 30 hr after single drug intake, and in this respect they are equivalent with the most modern registered retard theophylline preparations available on the market (13,14). No sex differences were observed comparing the pharmacokinetic parameters.

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