COMPARATIVE BIOAVAILABILITY AND IN VITRO IN VIVO CORRELATION OF TWO SUSTAINED RELEASE BRANDS OF THEOPHYLLINE: TABLETS AND PELLETS

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ABSTRACT

To investigate the influence of dosage forms on bioavailability, a randomized single-dose crossover study under fasting conditions was conducted using two commercially available sustained release products, Quibron SR tablets and Respro-SR pellets filled Capsules containing 300mg theophylline. A group of 12 healthy, male human subjects participated in this study. Serial blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. Theophylline was measured by high-performance liquid chromatography while absorption profiles were derived using Wagner-Nelson equation. The bioavailability of Quibron SR tablets was compared with Respro-SR pellets filled Capsules 300 mg using pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$. In addition, the 90% confidence interval (CI) for the ratio of logarithmic transformed C_{max} and $AUC_{0-\infty}$ was also used to determine bioequivalence. The T/R (test/reference) ratio of Quibron SR tablets was quite close to the prescribed limits of bioequivalence i.e. 80-125%. No statistically difference was observed between the log transformed $AUC_{0-\infty}$ (P=0.971) values as well as log transformed C_{max} values (P=0.854) indicating bio-availability and the extent of absorption of two brands were comparable. Moreover, the value of correlation coefficients for % *in vivo* absorption versus % *in vitro* dissolution of the two products was calculated to be 0.9533 for Respro-SR capsule and 0.9789 for Quibron-SR tablets.

Keywords: Sustained release, theophylline, bioavailability, *in vitro, in vivo* correlation.

INTRODUCTION

For a generic formulation of an existing drug, its bioavailability is generally evaluated relative to the standard formulation of the originator. For a formulation to be considered bioequivalent to a pioneer product there must be no statistical differences (as specified in the accepted criteria) between their plasma concentration-time profiles. Because two products rarely exhibit absolutely identical profiles, some degree of difference must be considered acceptable (James *et al.*, 2002). Oral sustained release dosages such as suspension, tablet or capsule require absorption prior to reaching the systemic circulation. The rate and completeness of absorption are variably decreased with formulations designed for delayed or slow release. Theophylline has been relegated to third-line therapy in asthma and is still useful in the management of patients with severe asthma and chronic obstructive pulmonary disease (Peter, 2003). Various bioavailability studies on sustained release preparations of theophylline has been conducted such as pellet formulation for once-daily dosage with a pellet preparation designed for twice-daily dosage (Keinke, 1989), four sustained-release from the Chilean market (Biagini *et al*, 1990), two Soviet sustained-release in comparison with TheoDur (Kiivet *et al.*, 1992), three slow-release in healthy Thai volunteers (Kanthawatana *et al*, 1996), slow-release tablet in children (West *et al*., 1994), and under Fasting and limited food conditions (Al-Ghazawi and Majali, 2000).

One of the major advantages of pellets is the gradual and to some extent more predictable emptying of from the stomach with small intra and inter-subject variations compared to tablets. Pellets usually get emptied rapidly from the stomach and can easily pass through the contracted pylorus. On the other hand gastric emptying of a single unit dosage form is essentially a random process with greater intra and inter-subject variations (Bechgaard, 1982). Therefore, in the present study, bioavailability and in vitro in vivo correlation of two oral sustained release dosage forms of theophylline were investigated. Two brands of theophylline were based on two different dosage forms one is single unit (Quibron SR tablets) while other is multiparticulate (Respro-SR pellets) containing 300mg theophylline. The other brands of tablets were also available but in different doses while no other brand of pellets was available.

MATERIALS

Theophylline (Bristol-Myers Squib Pakistan), paracetamol (GlaxoSmithKline Pakistan), methanol HPLC grade (Merck-Germany), acetonitrile HPLC grade (Merck-Germany), phosphoric Acid (Merck-Germany), potassium dihydrogen phosphate (Merck-Germany), isopropyl Alcohol (Merck-Germany), tetrahydrofuran (Merck-Germany), chloroform (BDH, England), phosphoric Acid, AR (BDH, England), Respro-SR® Capsules 300 mg (Searle, Pakistan; Batch No.0032, Mfg Date, 6/05, and Exp. Date. 5/08) and Quibron –T/SR® 300mg, (Bristol-

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METHODS

Drug content determination

Contents of two commercially available sustained release theophylline preparations such as pellets filled capsule, Respro-SR/300mg and tablet, Quibron T/SR/300mg were triturated separately in a mortar to a fine powder form. Powder mixture equivalent to 100mg of theophylline were transferred in 100ml volumetric flasks. The absorbance of both solutions was noted at wavelength of 272 nm using distilled water as blank.

In vitro dissolution studies

The in vitro dissolution profiles of capsules and tablets of theophylline were studied using USP Paddle method (Pharma Test, Hainburg, Germany). The test was performed in 900 ml distilled water maintained at 37 ± 0.5^0C and stirring speed of 100 rpm. Samples (5ml) were collected at 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours with an automatic fraction collector equipped with a piston pump (Watson Marlo, Stockholm, Sweden). The drug content in each sample was analyzed by dilution with the release medium at 272 nm using Shimadzu spectrophotometer (Tokyo, Kyoto, Japan). The amount of drug released matched the assay of drug content closely.

In vivo study protocol

Twelve (12) healthy non-smoking adult male subjects between 20 and 29 years old (Mean=22.8years, SD±2.8years), with heights from 150 to 170 cm (Mean=162.5cm, SD±4.0cm), and weighing from 50 to 63 kg (Mean =57.9Kg, SD±3.1Kg), participated in the study. Written informed consent was obtained from the volunteers after explaining the nature and purpose of the study. All were judged to be healthy on the basis of their blood and urine reports and were not receiving any medication during the study period. The protocol used was a conventional, two-way, split group, crossover study with 6 subjects in each of the two treatment groups. The preparations were administered according to the schedule shown below:

Group	Period	
	I	II
1	Respro-SR300mg Pellets	Quibron-T/SR 300mg Tablet
2	Quibron-T/SR 300mg Tablet	Respro-SR300mg Pellets

In the first trial period, each volunteer in first group was given one capsule of Respro-SR 300mg and the second group was given one tablet of Quibron-T/SR300 mg. After a washout period of two weeks, each volunteer then received the alternate product. Both the preparations were administered in the morning at 9.00 a.m. to fasting subjects with 240 ml of water in an attempt to reduce variability. Food and drinks were withheld for at least 2 hours after dosing. Blood samples of 5-ml volume were collected in vacutainers (containing sodium heparin as anticoagulant) at 0 (before dosing), 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours after dosing via an in-dwelling cannula placed in the forearm. Blood samples were also collected after 24 hours via direct puncture with 5-ml syringe. The blood samples were centrifuged for 10 min at 3500 rpm and the plasma was transferred to new glass tubes and kept frozen until analysis.

Analysis of plasma theophylline concentration

The plasma samples were analyzed using a reversed-phase high-performance liquid chromatographic (HPLC) method. The HPLC system comprised of an Agilent Technologies 1200 pump (Germany), an Agilent Technologies UV detector (Germany) and a Rheodyne 7725 sample injector fitted with a 20 µl sample loop. The detector was operated using a wavelength of 272 nm. Data Processing Modular was connected with the detector and the signals of detector were analyzed by HPLC Software, Agilent Chemstation.

A ODS (octadecyl silane) C18 column (10µm, 250x 4.6 mm ID) fitted with a guard column was used for separation. The mobile phase consisted of 0.05M potassium dihydrogen phosphate: methanol: tetra-hydrofuran (83.2%: 15%: 1.8%). The pH of mobile phase was adjusted to 4.0 with phosphoric acid. The mobile phase was filtered through a 0.45µm membrane filter (Sartorius U.S.A) and was then degassed by ultrasonication. Analysis was run at a flow rate of 1.0 ml/min and quantification was by peak height.

Prior to injection, theophylline was extracted from the plasma samples according to the following procedure: 250 µl of the plasma sample was measured into an Eppendroff microcentrifuge (Greiner lavortechnik-Germany) tube, followed by adding 50 µl of paracetamol internal standard solution (100µg/ml) and 1.0 ml of 8:2 chloroform- isopropyl alcohol extracting solvent. The mixture was then vortexed for 1 min by using a vortex mixer (Seouline BioScience-Korea), and centrifuged at 10,000 rpm for 5 min by centrifuge machine (Kubota, Japan). After centrifugation solvent was dried under a gentle stream of nitrogen at 45^{o}C. The residue was reconstituted with 100 µl of mobile phase and 20 µl injected into column.

Standard curve was constructed to encompass anticipated range of plasma theophylline concentration found in healthy volunteers. Blank plasma was spiked with theophylline drug solutions to give concentrations of 30, 15, 7.5, 3.75, 1.87µg/ml. The standard was stored at –20^{o}c in the glass bottles. Recovery, within-day and between-day precision and accuracy studies (n=6) were carried out using these plasma standards. In addition, detector linearity was determined with theophylline standard

Table 1: Individual numerical values of AUC 0-∞, C_{max} and T_{max} from Quibron SR tablets and Respro SR capsules

Subject	Quibron-SR 300mg tablets			Respro-SR 300mg Capsules (pellets)		
	AUC 0-∞	Cmax	T_{max}	AUC 0-∞	Cmax	T_{max}
	(µg.hr/ml)	(µg/ml)	(hr)	(µg.hr/ml)	(µg/ml)	(hr)
S1	128.3	5.6	6.00	130.8	6.1	6.00
S2	106.8	7.1	5.00	108.8	7.5	7.00
S3	97.7	6.4	7.00	90.0	6.1	7.00
S4	105.7	6.7	6.00	125.3	8.7	6.00
S5	90.0	4.2	6.00	85.0	3.3	6.00
S6	65.6	7.7	7.00	49.6	5.8	8.00
S7	117.2	7.5	7.00	79.9	5.4	7.00
S8	126.2	9.3	6.00	134.1	9.3	6.00
S9	109.6	6.5	5.00	115.6	7.3	6.00
S10	47.8	4.0	4.00	69.2	4.0	6.00
S11	101.3	9.6	5.00	71.5	8.8	5.00
S12	109.8	7.4	7.00	114.0	7.4	6.00
Mean	100.5	6.8	5.9	97.8	6.6	6.3
SD	23.48	1.70	1.00	27.37	1.88	0.78

Table 2: Individual pharmacokinetic values (Ke, t1/2 and Vd) of theophylline in Quibron SR tablets and Respro SR capsules

Subject	Quibron-SR 300mg tablets			Respro-SR 300mg Capsules		
	Ke	t 1/2	Vd	Ke	t 1/2	Vd
	(hr^{-1})	(hr)	(L/Kg)	(hr^{-1})	(hr)	(L/Kg)
S1	0.048	14.4	0.854	0.036	19.3	1.123
S2	0.071	9.6	0.681	0.076	9.1	0.624
S3	0.134	5.14	0.375	0.244	2.8	0.223
S4	0.083	8.3	0.600	0.093	7.4	0.451
S5	0.037	18.2	1.581	0.034	20.5	1.821
S6	0.097	7.1	0.748	0.107	6.4	0.897
S7	0.085	8.2	0.510	0.086	8.0	0.739
S8	0.104	6.7	0.415	0.106	6.5	0.384
S9	0.067	10.3	0.658	0.074	9.3	0.566
S10	0.076	9.1	1.422	0.069	9.9	1.083
S11	0.079	8.7	0.668	0.048	14.4	1.561
S12	0.078	8.8	0.673	0.056	12.3	0.902
Mean	0.080	9.5	0.8	0.086	10.5	0.9
SD	0.025	3.5	0.4	0.056	5.3	0.5

solutions prepared in distilled water over a concentration range of 10-30µg/ml.

Pharmacokinetic parameters analysis

The most common pharmacokinetic parameters such as total area under the plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were estimated from the plasma concentration-time profiles of the two preparations for each volunteer. The C_{max} and T_{max} values were obtained directly from the plasma-concentration data (Weiner, 1981). The $AUC_{0-\infty}$ was calculated by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling time to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula and the latter by dividing the last measurable plasma drug concentration with the apparent elimination rate constant (k_e). In all cases, the $AUC_{t-\infty}$ was found to be less than 10% of the $AUC_{0-\infty}$. The k_e was estimated from the terminal slope of

Table 3: Comparison of individual numerical values (AUC 0-τ) of two brands

Subject	Respro-SR Capsules (R)	Quibron-SR Tablets (T)	T-R	T/R	ln(T/R)
	AUC 0-τ (h.ug/ml)	AUC 0-τ (h.ug/ml)			
S1	102.9	101.3	-1.6	1.0	-0.02
S2	88.5	92.3	3.8	1.0	0.04
S3	82.8	91.2	8.4	1.1	0.10
S4	108.0	89.2	18.8	0.8	-0.19
S5	55.58	63.7	8.1	1.1	0.14
S6	45.8	59.0	13.2	1.3	0.25
S7	67.0	97.4	30.4	1.5	0.37
S8	119.4	111.8	-7.6	0.9	-0.07
S9	92.8	84.6	-8.2	0.9	-0.09
S10	54.0	40.2	-13.8	0.7	-0.30
S11	109.8	85.4	-24.4	0.8	-0.25
S12	86.3	92.8	6.5	1.1	0.07
Mean	84.4	84.1	-0.3	1.0	0.01
SD	24.1	20.1	15.3	0.2	0.2

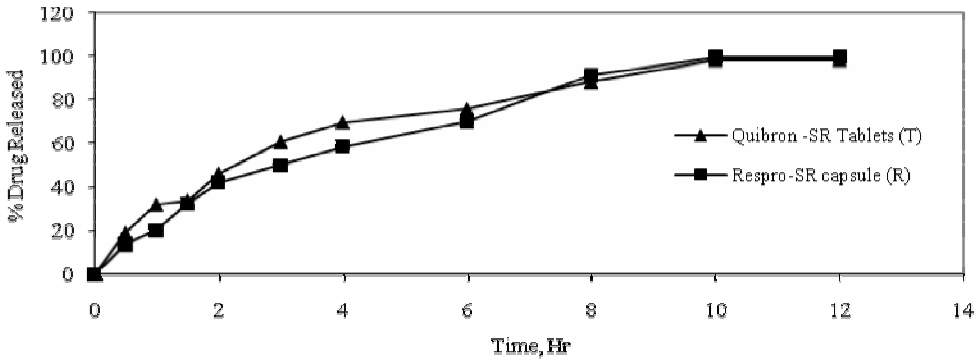

Fig. 1: *In vitro* release profiles of theophylline from two commercially available sustained release products.

the individual plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression (Gibaldi and Perrier, 1982). On the other hand, the elimination half-life ($t_{1/2}$) was calculated from the quotient ln 2/ k_e, while the apparent volume of distribution (Vd) was calculated as Dose/($AUC_{0-\infty}$ x k_e) .The *in vivo* absorption profiles of the formulations were also calculated from the individuals plasma concentration versus time data using Wagner-Nelson method (1964).

STATISTICAL ANALYSIS

The calculated values of the parameters, $AUC_{0-\infty}$, C_{max}, t½, k_e and Vd obtained with the two preparations were analyzed statistically using an analysis of variance (ANOVA) procedure which distinguished effects due to subjects, periods, and treatment (Wagner, 1975). The $AUC_{0-\infty}$ and C_{max} values were logarithmically (log) transformed prior to the analysis. On the other hand, the T_{max} values of the two preparations were analyzed using the Wilcoxon Signed Rank Test for paired samples. A statistically significant difference was considered when $P< 0.05$. In addition, the 90% confidence interval of the ratio of Logarithmic transformed AUC 0-∞ as well as C_{max} of the test formulation over those of the reference capsules was also determined.

RESULTS AND DISCUSSION

In vitro evaluation

In vitro dissolution profiles of two brands of theophylline, Quibron-SR tablets (test) and Respro-SR filled pellets in capsules (Reference) are shown in fig. 1. Slight differences in the release profiles of the two formulations are apparent. Theophylline release from both the formulations was more than 90% during 10 hours testing time, but the release rate constant of Quibron-SR tablets was slightly lower as compared to Respro-SR capsules. Moreover, when *in vitro* release profiles of two formulations were fitted in f_2 equation (Moore and Flanner, 1996) for similarity, the value obtained was above 50 indicating both the formulations are comparable.

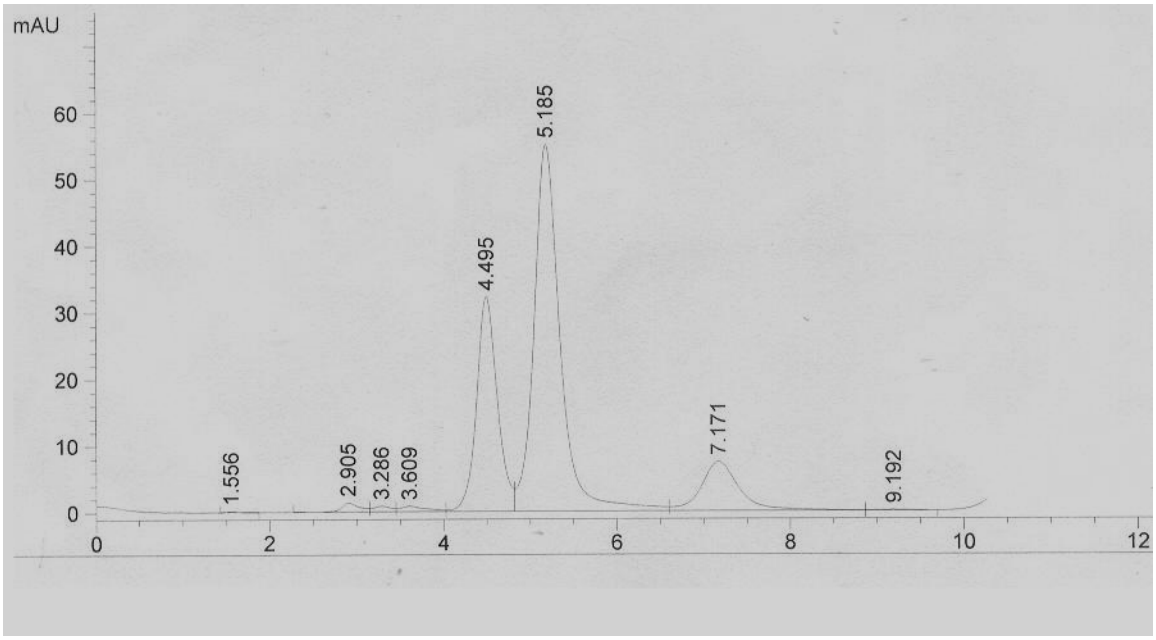

Fig. 2: HPLC chromatogram of plasma sample taken from a healthy subject at 4 hours after administration of Quibron-SR 300mg tablets.

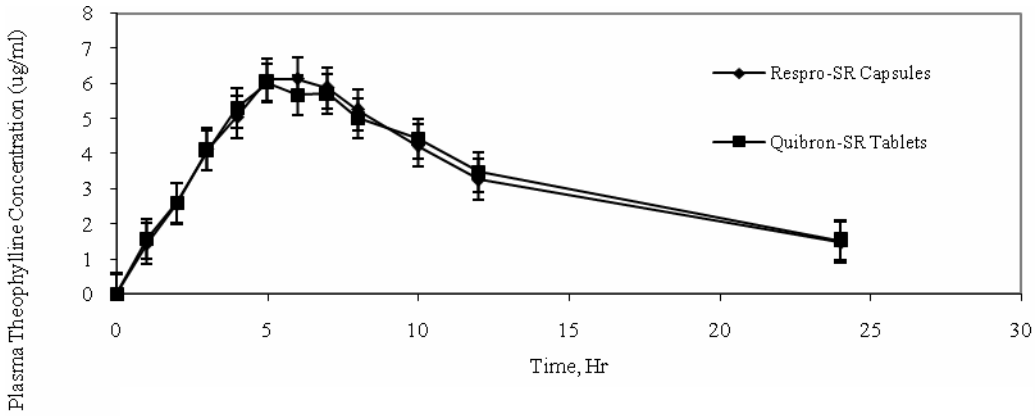

Fig. 3: Mean plasma theophylline concentration versus time profiles of Respro capsules and Quibron tablets Mean ± SEM, N=12.

Analysis of theophylline in plasma

Several HPLC methods have been reported for the analysis of theophylline and some of the methods involve liquid-liquid extractions with slight differences in the extracting solvent mixtures, the mobile phase, the size of the column and the total time for analysis. In the present study, a single stage extraction method with minimum extraction time and analysis time was developed from the reported methods (Yuen, *et al*, 1997; Shishoo and Savale, 1999). The reported method was slightly modified by changing the composition of mobile phase consisting of 0.05M potassium dihydrogen phosphate: methanol: tetrahydrofuran (83.2%: 15%: 1.8%) was utilized in the present study for good separation of compounds with sharp peaks within 10 minutes.

The chromatogram of plasma samples taken from a volunteer at 0 hour and 4.0 hour after dosing of Quibron-SR tablet is presented in the fig. 2. The retention time of theophylline and internal standard (paracetamol) is 5.1 and 4.4 respectively. The blank sample was clean and no interfering peak was observed at the retention times of theophylline, and there is no interference between the peaks of theophylline and internal standard. The extraction recovery of theophylline was determined by comparing the peak height obtained by direct injection of standard aqueous solutions to those obtained after the plasma extraction procedure. The recovery values for theophylline were more than 90% at 1.87µg/ml and 15.0µg/ml. The sensitivity of the assay method was approximately 0.5µg/ml.

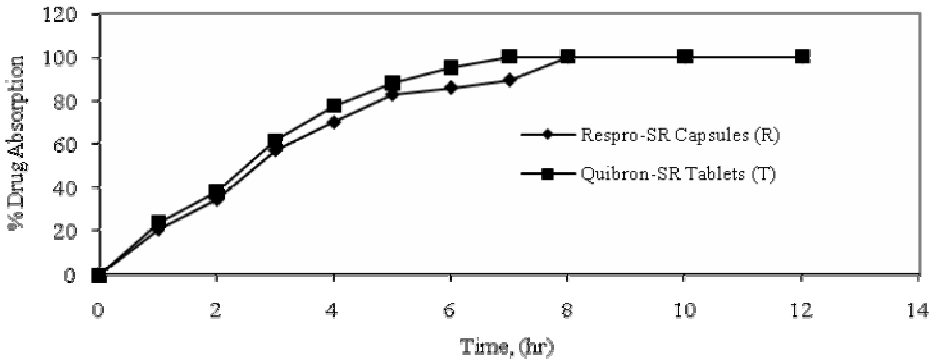

Fig. 4: Mean *in vivo* theophylline absorption versus time profiles of pellets filled Respro-SR capsules and Quiron-SR tablets (N=12).

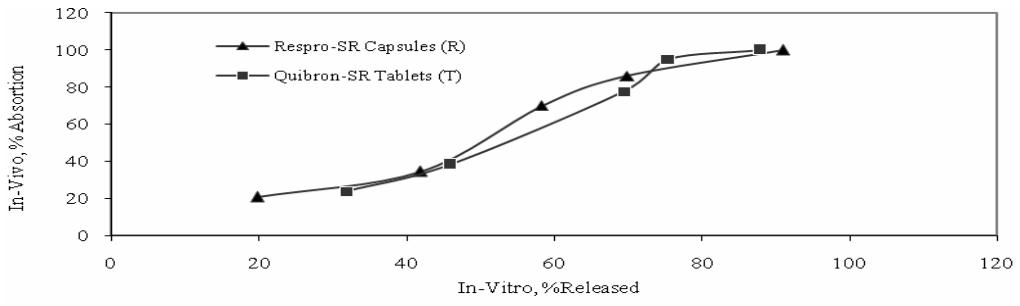

Fig. 5: *In vivo* theophylline % absorption versus *in vitro* % released profiles from Respro-SR capsules® and Quiron-SR tablets (T).

In vivo performance of test and reference formulations

Mean plasma theophylline concentration versus time profiles of Respro-SR pellets filled capsules and Quibron-SR tablets is shown in fig. 3. Slight differences in their mean plasma profiles are apparent and the two formulations are seemed to be comparable. Both in vivo profiles are reflective of a slow and sustained rate of drug absorption. Plasma concentrations of theophylline were detectable during 24 hours from the two preparations. Though lag time in absorption has been observed by Peh and Yuen (1996) with their controlled release preparation but no lag time in the plasma concentration of the two formulations were noticed. There was rapid increase in the plasma concentration and reaching maximum at approximately 6-7 hours after dosing, being typical that obtained with sustained release preparations. The mean in vivo theophylline absorption profiles versus time of two formulations calculated by using Wagner-Nelson method (1964) are depicted in fig. 4. Both the profiles were comparable and no significant difference in their absorption was found.

The individual numerical values of pharmacokinetic parameters $AUC_{0-\infty}$, C_{max}, and T_{max} obtained with pellets filled Respro-SR capsules and Quibron-SR tablets are presented in table 1. Whilst, the values of k_e, $t_{½}$ and Vd of the two formulations are given in table 2 and were closely similar and were not significantly different statistically.

The mean T_{max} values for Respro-SR (6.3±0.78 hours) was similar compared to Quibron-SR (5.9±1.00 hours) indicating similar absorption rate. This value is very close to the previously reported values of T_{max} after a single oral dose of 300 mg in healthy subjects (Al-Gahzavi *et al*, 2000). Non parametric analysis using the Wilcoxon test showed no significant difference between the T_{max} values of Respro-SR capsules and Quibron-SR tablets (p=0.129). In the present study, C_{max} for Respro-SR capsules was found to be 6.6±1.88 µg/ml compared to 6.8±1.70 µg/ml of Quibron-SR tablets. C_{max} of the reference Respro-SR was slightly higher than Quibron-SR. However, no significant difference (P=0.651) was found between the values of C_{max} of both the brands.

$AUC_{0-\infty}$ for Respro-SR capsules and Quibron-SR tablets were 97.8±27.37µg. hr/ml and 100.5±23.48 µg. h/ml, respectively. No statistically difference was observed between the log transformed $AUC_{0-\infty}$ (P=0.971) values as well as log transformed C_{max} values (P=0.854) of the preparations indicating that the bioavailability and the

extent of absorption between the two preparations were comparable. In addition, the 90% confidence interval (CI) for the ratio of the log transformed $AUC_{0-\infty}$ values of the Quibron-SR over those of Respro-SR was estimated to be between 0.978 and 1.032, the ratio of the log transformed C_{max} values of Quibron-SR tablets over those of Respro-SR capsules was estimated to be between 0.937 and 1.069 which is within the acceptable bioequivalence limits of 0.80-1.25 (USP 24, 2000). To facilitate bioequivalence comparisons, pharmacokinetic parameters for each volunteer were displayed in parallel for the two formulations. For AUC_{0-t} in particular, the difference (T-R), ratio (T/R) and log of ratio (log T/R or Ln T/R) between the test and reference values were tabulated side by side for all the subjects and shown in table 3.

The elimination rate constant, K_e of Respro-SR capsules and Quibron-SR tablets were 0.086±0.056 h^{-1} and 0.080±0.025 h^{-1} respectively. Similarly, the volume of distribution (V_d) of Respro-SR capsules and Quibron-SR tablets were 0.9±0.5 L/Kg and 0.8±0.4 L/Kg, and no statistically difference ($P>0.05$) in K_e and V_d values of both the brands were found.

The plots of % *in vivo* absorption versus the % in vitro dissolution of the two formulations are shown in fig. 5. It is apparent that the two plots are divergent in nature. The slower rate of absorption was observed with Quibron-SR tablet compared to Respro-SR capsule. In contrast, in vitro dissolution profiles indicated slower release rate in Respro-SR capsule instead of Quibron-SR tablets. This is attributed to the difference in the gastrointestinal transit time for tablets and pellets. As the pellets filled capsules can easily pass through stomach even when the sphincter is closed. Therefore the tablets might have variable GI transit time compared to pellets. However, statistically correlation was observed between the in vivo absorption and in vitro dissolution data for both brands and appeared to be relatively linear. The value of correlation coefficients was calculated to be 0.9533 for Respro-SR capsule and 0.9789 for Quibron-SR tablets.

CONCLUSION

Pharmacokinetic values of theophylline obtained from Respro-SR capsules (reference) and Quibron-SR tablets (test) were comparable in the extent of bioavailability ($AUC_{0-\infty}$ and C_{max}) and in the rate of absorption (C_{max} and T_{max}). The *in vivo* absorption profiles of the two formulations were also comparable and no significant difference in their absorption was found. In addition, no statistical difference was observed between the log transformed data of $AUC_{0-\infty}$ and C_{max} ($P>0.05$) in comparing the two products by analysis of variance. Moreover, the 90% confidence interval for the ratio of the log transformed $AUC_{0-\infty}$ and C_{max} values of Quibron-SR tablet over those of Respro-SR capsules were within the acceptable bioequivalence range of 0.80 and 1.25. Therefore, it can be concluded that two different dosage forms had little effect on the bioavailability of theophylline.

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