In vivo Evaluation of a Theophylline Matrix Tablet Formulation

Magda S. Sokar*, Viviane F. Naggar, Safaa S. El Gamal

Department of Pharmaceutics, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt

*Corresponding author:

Tel.: +203-48-71317; Fax: +203-48-73273

E-mail address: magdasokar@yahoo.com (Magda S. Sokar)

Abstract

In this study, the in vivo testing of the selected colon-targeted compressed coated tablet formulation F2 (Treatment A), prepared in our previous work, was compared with that of the tablet core as control (Treatment B) on 6 healthy non-smoker male volunteers in a crossover design study was performed. Saliva samples were collected at different time intervals over a period of 24 hours and assayed for theophylline by HPLC. ANOVA for a crossover design revealed significant differences between the two treatments with respect to T_{max}, C_{max} and log AUC_{0-24}.

High coefficients of determination for the in vitro AUC_{0-t} release profiles (in the previous study) and the in vivo AUC_{0-t} obtained from the saliva concentration time profiles, for both treatments A and B, were observed by applying the quadratic model in case of treatment A and quadratic or cubic model in case of treatment B.

Keywords: colon-targeted, compressed coated tablet, crossover design, saliva & tablet core

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Introduction

A theophylline (TH) formulation coated with 500 mg pectin proved to be a successful colon-targeted formulation, giving a neglected drug release in the stomach and not more than 2.9% in small intestine. It was therefore, selected for further in vivo study in human volunteers.

Literature has established that TH can be efficiently absorbed throughout the gastrointestinal tract, specifically from the large bowel (1-2) and colon (1,3). In addition, it has been shown that food and drugs that alter gastrointestinal tract transit time can affect the rate but not the extent of TH absorption (1,4-5). TH plasma concentration with time after therapeutic doses, can be well described by linear kinetics (6). However, at doses exceeding 10mg/kg, it shows nonlinear kinetics (7). Protein binding in plasma is about 40% at a therapeutic plasma concentration of 12-15µg/ml (8). The plasma half–life was observed in the range of 3-16 hours (9). TH has a volume of distribution (v_d) of about 0.5 liter/kg and a

plasma clearance of about 0.5 to 2 ml/min/kg in normal subjects. It is rapidly and widely distributed throughout tissues (8).

In spite of normally linear kinetics in the therapeutic dose range, TH $t_{1/2}$ varies from roughly 3 to 20.7 hours in healthy adults and 1.4-7.9 hours in asthmatic children. In premature primary apnoea, a range of 12.6-29 hours has been reported (10).

Many factors can influence TH pharmacokinetics such as age, smoking, drug interactions and stress. In newborn infants, mean half-life ($t_{1/2}$) is over 24 hours. There is a linear relationship between age and $t_{1/2}$ in infants up to 6 months of age; over 6 months mean $t_{1/2}$ is 3.7 ± 1.1 hours. Adult nonsmokers with uncomplicated asthma show a mean $t_{1/2}$ of 8.7 ± 2.2 hours. In smokers (1 to 2 packs per day) $t_{1/2}$ is 4-5 hours. After cessation of smoking, normalization of TH pharmacokinetics may not occur for 3 months to 2 years (10-11).

Protein binding decreases in neonates and in subjects with hepatic cirrhosis (8) In older adults with chronic obstructive pulmonary disease, corpulmonale, or other causes of heart failure, and liver pathology, $t_{1/2}$ may exceed 24 hours (10).

Reduced clearance and increased toxicity have been reported in congestive heart failure. The mean half-life values increased slightly in obese patients, while the volume of distribution (v_d) was not altered when based on the total body weight (TBW) (10-11).

The variations of plasma half-life in adults and marked decrease in children is probably related to differences in metabolic activity in individual subjects (12).

The metabolism of TH is shown in Figure 1. By N-demethylation, 3-methylxanthine is formed. It is one-third to one-fifth as potent as TH. However, its excretion in the urine is more rapid than its hepatic formation so that it is unlikely to contribute to the pharmacological effect (4,8). In adults, about 13% of the dose is excreted in the urine in 24 hours as unchanged drug, with about 15% as 3-methylxanthine, 35 to 50% as 1,3-dimethyluric acid and in about 20% as 1-methyluric acid (8).

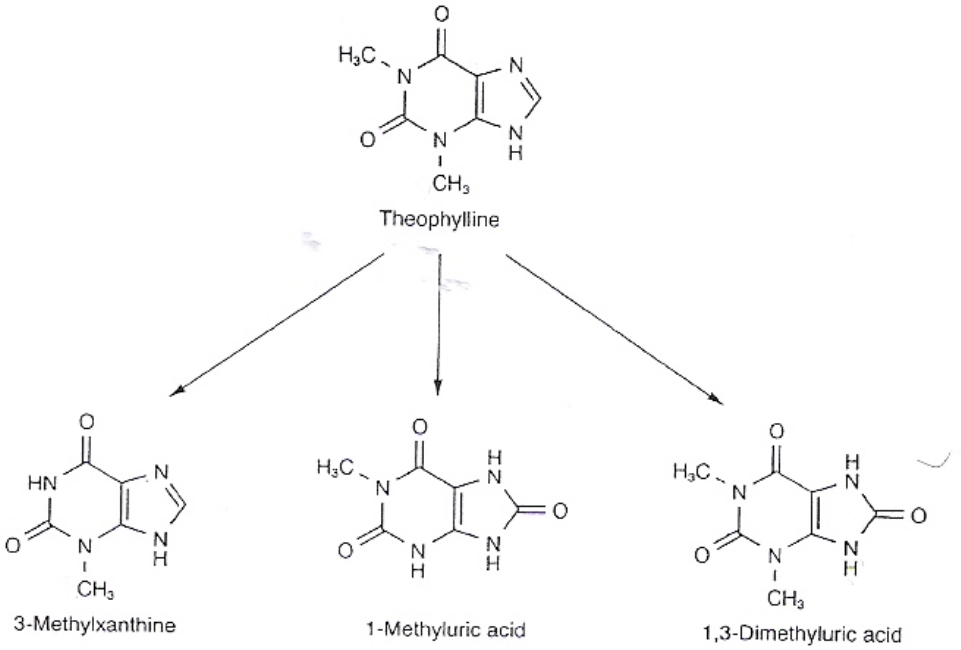

Figure 1: Metabolic pathway of theophylline

TH disposition following oral administration can be described by an open two-compartment pharmacokinetic model with an average plasma half-life for β-phase disappearance of 4.4 hours, and a half-life for distribution (α-phase) of 0.12 hours. There are no statistically significant differences in the pharmacokinetic parameters between normal volunteers and asthmatic subjects (12). It was shown that

asthmatic children evidenced significantly shorter β-phase half-lives than adults with a mean of 2.65 hours compared to 4.4 hours for the adult population (13) .

It is clear from the previously reported data on the pharmacokinetics of TH, that the drug would be preferably administered as modified release formulations for chronic asthmatic cases and for children, especially the young ones, who tend to metabolize TH at a faster rate than adults (14).

Although the most effective initial treatment of acute symptoms is an inhaled or parenteral β_2 agonist, the bronchodilator effects of TH are of value in the treatment of prolonged attacks. Oral TH is widely used when there are no facilities for the parenteral route and when the technical difficulties limit the use of inhalation therapy in aged patients.

Only in recent years has TH therapy became dependent on pharmacokinetic management. The effective plasma concentration range for TH is rather narrow. Although improvement in pulmonary function was reported at blood levels 5, 10 and 20 µg/ml, it is generally agreed that 10-20 µg/ml is the desirable range (4,15). However, clinical trials have suggested that the drug can produce significant suppression of the late asthmatic response in doses below the normal therapeutic level (16).

Purpose

In this study, in vivo testing is performed on healthy male volunteers in order to investigate the effect of the selected compressed coated tablet intended for colon targeting on the bioavailability of TH as a prophylactic therapy to prevent early morning symptoms in chronic asthma. Comparison is done with the tablet core of the prepared formulation.

Materials and Methods

Anhydrous theophylline powder (TH), Boehringer Ingelheim KG, Ingelheim , Germany. Dextran grade C (Dc) of molecular weight 60,000 - 90,000; Biochemical, BDH Chemical Ltd., Poole, England. Microcrystalline cellulose of chromatographic grade, (Cellulose D. Avicel-mikrokristallin (MCC), Riedel-De Haenag Seelee-Hannover. Pectin, Unipectine UHM73C, Degussa Texturant Systems, France, SAS. Caffeine anhydrous powder, KNOLL AG. Ludwigshafen, Germany. Acetic acid glacial, Scharlau Chemie S.A. Barcelona, Spain. Trichloroacetic acid, Fluka Chemie AG, Buchs, Switzerland. Methyl alcohol; HPLC grade, Romil Chemical Ltd., Shepshed, Leices, England. All other chemicals were of analytical or pharmaceutical grade.

METHODS

Preparation of theophylline tablet core and compression coated tablets

All powders were sieved and fractions corresponding to particle size range 71µm-1.25mm were used. The tablet core was prepared by direct compression of geometrically mixed powders of the drug (TH), Dextran C and Avicel (2:1:2) by means of 6 mm round flat faced punches. Before compression, a content uniformity test was done on a suitable number of samples accurately weighed taken from the powder mix to contain the equivalent of 500 mg TH.

The tablet core was compressed coated with 500 mg pectin using 12 mm concave faced punch. The tablet core was used as control to be compared with F2 in the crossover design.

In vivo study

Design of the in vivo study

Six healthy adult male volunteers capable of informed consent, weighing between 72 and 98 kg (mean = 87 kg) and ranging in age between 21 and 48 years (mean = 37.8 years), participated in the study. Moreover, an agreement of a Committee headed by the Dean of Faculty of Pharmacy, Alexandria University was obtained prior to the in vivo experiment. None of the subjects was a smoker. No medication was permitted for one week prior to and throughout the testing period. Subjects were not allowed to take any food or drinks other than water for 12 hours prior to and 5 hours following tablet administration. Food and liquid intake after the first 5 hours, was limited to non xanthine-containing

products. Participants were also forbidden from any strenuous activities during the first 8 hours after tablet administration.

Tablet administration was performed according to the crossover design shown in Table 1. Two weeks washout period followed each treatment.

Table 1: Crossover design for subjects participating in the TH bioavailability study

Subject	Age (yr)	Body weight (kg)	Height (cm)	Week 1	Week 2
1	22	72	170	B	A
2	21	75	165	A	B
3	43	88	170	A	B
4	45	95	180	B	A
5	48	98	183	B	A
6	48	94	175	A	B
Mean	37.8 ± 11.7	87 ± 10.0	173.8 ± 6.2		

A is compressed coated tablet (F2)
B is the tablet core (TH, Dc, Avicel 2:1:2)

Treatment A is the coated TH tablet formulation which is the tablet core compressed coated with 500 mg pectin. Treatment B is the tablet core containing 50 mg TH, 25 mg Dc and 50 mg Avicel.

Two tablets of each of the control and treatment were given to the volunteers. With the aid of 1 or 2 crystals of citric acid placed on the tongue, approximately 5 ml saliva were collected in dry centrifuge tubes, at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours following tablets administration. The samples were stored at -20°C, until analysis.

Analysis of saliva samples

Saliva samples were analyzed using the reversed phase HPLC method previously reported for TH analysis (17-18) but with some modifications.

The samples were treated as follows: The collected sample was first vortexed for 1 minute then centrifuged (Centrifuge, type H-13 A, Kokusan Ensinki Co., Ltd., Tokyo, Japan) for 15 minutes at 3000 rpm. Twenty µl of the internal standard solution (caffeine 100 µg/ml in methanol) were added to 1 ml of the centrifuged saliva followed by 200 µl of 50% solution of trichloroacetic acid. The tubes were vortexed for 1 minute and then centrifuged for 20 minutes at 3500 rpm. Twenty µl of the supernatant were injected onto the column (Liquid chromatograph consisting of a solvent pump (Waters 501). reverse phase column (µ Bondapack C_{18}, 3.9 x 300 mm Waters) and a UV detector (Waters model 441); Millipore Corp., Waters Chromatography Division, Milford, Ma USA).

Spiked standards were prepared by adding an aliquot of a methanolic solution of TH, 100 µg/ml, to 1 ml blank saliva to cover a concentration range from 0.25 to 5 µg/ml.

The chromatographic conditions were: 25% methanol and 75% acetic acid (0.8 g%) as mobile phase, at flow rate of 2ml/min, wavelength was 272 nm. Sensitivity, not less than least limit of quantification, found to be 0.25 µg/ml.

Under these conditions the retention times of TH and caffeine were 2.07 ± 0.3 and 3.73 ± 0.3 minutes respectively (Figure 2).

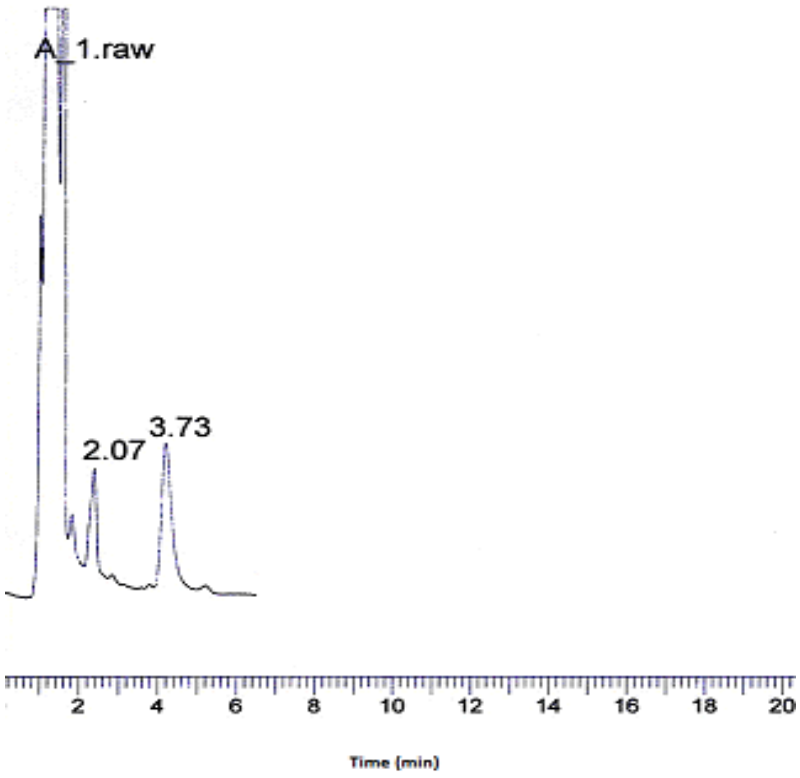

Figure 2: Chromatogram of TH and caffeine in saliva

Validation of the assay methodology

Recovery values from saliva were obtained by comparing the detector response to pure authentic standards with the response from equivalent amounts added to, and recovered from the saliva. At least three standards were injected on each analysis day among the samples analyzed. The ratio of TH peak area to that of the internal standard was calculated. The regression equation was then obtained relating TH concentration in saliva to peak area ratio, and was used to calculate concentration in the analyzed samples.

Interday and intraday precision was assessed by examining the coefficients of variation, calculated for standards assayed on different days or on the same day respectively. The amounts found by refitting the ratios from the calibration standards in the derived regression equation were evaluated, and their means, standard deviations and coefficients of variation were calculated.

Calculation of some pharmacokinetic parameters

The calculated parameters include the following: area under the salivary TH concentration time curve from zero time to 24 hours (AUC_{0-24}), maximum saliva TH concentration reached (C_{max}), time needed to reach C_{max} (T_{max}).

All the above mentioned parameters were calculated using Stripe computer program (19) (University of lllinois, Chicago), except for C_{max} and T_{max} which were obtained from the saliva concentration time curves.

AUC_{0-t} is the area under the saliva concentration time curve from zero to time t calculated by the trapezoidal method (20) for correlation with the AUC_{0-t} obtained from the in vitro release profile at the same time intervals.

Statistical analysis of results

According to the recommendations of the USP 30, ANOVA tests for a crossover design were carried out to assess the effects of sequence, subjects within sequence and periods on C_{max}, T_{max} and on

the log transformed data of AUC_{0-24}. All the effects were tested against the mean square term for subjects within sequence (21). The analysis was done using Mixed Model Least-Squares and Maximum Likelihood Computer Program Pc-2 (22).

RESULTS AND DISCUSSION

The bioavailability study was performed by determining saliva TH concentrations. The existence of a good correlation between saliva and plasma levels of the drug has been proven (23-25). Also correlation of serum and saliva TH concentration after administration of sustained release preparations has been reported (26). The ratio for TH saliva/plasma levels was found to be 0.5 (26-28).

The study of Shah and Riegelman (12,28) indicated that the disappearance of TH from saliva paralleled that from plasma over the normal therapeutic concentration range. This may lead to a simplified method for monitoring TH concentration in clinical pharmacokinetic studies for patients undergoing chronic therapy.

The average salivary levels of TH following oral administration of a compressed coated colon targeted formulation of TH (treatment A) were compared with those of its tablet core (treatment B) in a crossover design (Table 1). All preparations were well tolerated by the volunteers.

Validation parameters of the assay were limited to TH recovery, interday, intraday precision and linearity data. The recovery was 98.8 ± 4.57% from spiked saliva samples. Data for interday, intraday precisions and their linearity are shown in Tables 2 & 3. The suitability of the method for determining saliva TH levels was confirmed by the low coefficients of variation and the high coefficients of determination.

Table 2a: Intraday precision data

TH conc.in saliva (µg/ml)	Mean conc. found (µg/ml)	Coefficient of variation (CV%)
0.5	0.503 ± 0.02	4.0
1	1.453 ± 0.03	2.1
2	2.122 ± 0.09	4.2
3	3.034 ± 0.137	4.5
4	3.664 ± 0.013	0.4
Mean CV%		3.04

Table 2b: Linear regression data for TH spiked saliva standards

Regression equation	Coefficient of determination (R^2)
Y = 0.3957x + 0.1621	0.9324
Y = 0.2922x + 0.1620	0.9265
Y = 0.2731x + 0.166	0.9317
Y = 0.2814 + 0.1868	0.9117

Table 3a: Interday precision data

TH conc.in saliva (µg/ml)	Mean conc. found (µg/ml)	Coefficient of variation (CV%)
0.5	0.520 ± 0.029	5.6
1	1.077 ± 0.020	1.9
2	2.265 ± 0.05	2.2
3	3.104 ± 0.15	4.8
4	4.011 ± 0.202	5.0
5	4.892 ± 0.274	5.6
Mean CV%		4.2

Table 3b: Linear regression data for TH spiked saliva standards

Regression equation	Coefficient of determination (R^2)
Y = 0.5888x + 0.0834	0.9806
Y = 0.6966x – 0.002	0.9966

Figure 3 gives the mean saliva TH concentrations for both treatments tested. It is worthy to note that, the mean saliva levels following treatment A were lower than those following treatment B.

Table 4 shows the mean pharmacokinetic parameters of TH. The individual and mean C_{max} values following treatment A, were lower than the values following treatment B, the mean C_{max} value of A being about 50 % that of B. Also T_{max} value was significantly lower in case of treatment A.

Table 4: Some pharmacokinetic mean parameters of TH following administration of treatments A and B

Parameter	B	A
$AUC_{0\text{-}24}$ h µg/ml	19.7	8.5
C_{max} µg/ml	0.96	0.48
T_{max} h	8	12

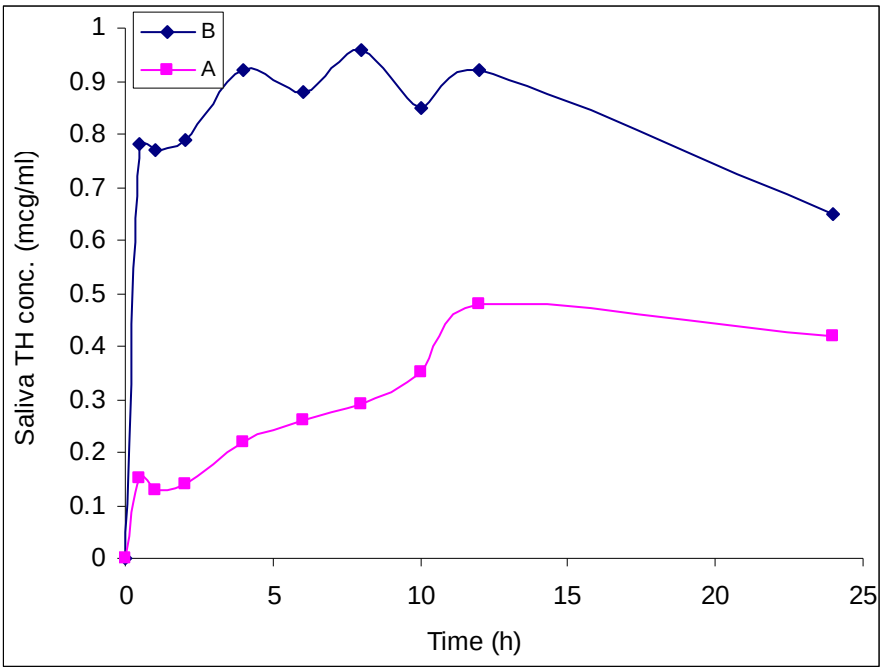

Figure 3: Mean saliva TH concentrations following administration of treatments A and B

In a previous report (26), using 250 mg TH powder in cachet, the average peak salivary concentration occurred at about 2.2 hours and the C_{max} was about 1.44 µg/ml after normalization to 100 mg drug. In another study (29), also using 100 mg TH in cachets, the corresponding values were 2.2 ± 0.8 hours and 1.22 ± 0.7 µg/ml respectively. Moreover, in a previous report (30) concerning in vivo evaluation of fast release double layer TH suppositories containing 100 mg drug, the mean salivary drug concentrations were found to be comparable with the results of the present study.

Different salivary TH profiles for treatments A and B were also seen. This probably reflects their different dissolution rates. Maximum peak TH level with treatment A was lower than that with treatment B and the time to reach the peak level was longer. However, much of the differences are arbitrary because of the prolonged flatness of TH peak level especially with treatment A.

Moreover, some fluctuations in mean salivary TH concentration profile occurred (31) (especially in treatment B) which may influence the accuracy of determining C_{max} and T_{max}. This may be due to differences in thickness of the mucosa in each part of the colon which may affect its permeability to the drug. Double peaks have been observed in the plasma level curves of several other drugs such as acetaminophen (32), aspirin (33), furosemide (34), penicillamine (35) and flurbiprofen (36) following oral administration. Double peaks have also been detected from plasma level-time profiles after administration of TH syrup to fasting healthy volunteers. In another study, an attempt was done to explain the occurrence of double peaks through a model proposed by Oberle and Amidon (31).

It is worthy to note that treatment A demonstrated lower AUC compared to treatment B. $t_{1/2}$ values (calculated by Stripe computer program) of treatments A and B were markedly different and were not reliable (37) as the elimination phase was not complete. Moreover, wide patient-to-patient variation with half lives has been reported (12) which is probably related to the differences in metabolic activity in individual subject.

An in vitro in vivo correlation (IVIVC) should be evaluated to demonstrate the predictability of the in vivo performance of a drug product from its in vitro dissolution characteristics. The objective of

developing an IVIVC is to establish a predictive mathematical model describing the relationship between an in vitro property and a relevant in vivo response.

The in vitro dissolution profiles were compared with the mean saliva TH concentrations for each of treatment A and B by plotting values of AUC_{0-t} calculated from the saliva concentration time profiles versus the AUC_{0-t} values obtained from the in vitro release profiles at the same time intervals. The correlation was estimated within 24 hours for both treatments. This kind of correlation was quite important since it represents a point to point relationship between in vitro dissolution and in vivo input rate of the drug from the dosage form. Thus, an in vitro dissolution curve can serve as surrogate for in vivo performance.

It is considered that in vitro dissolution test conditions can appropriately reflect the dissolution in the GIT, when the values of coefficient of determination are 1 or nearest to 1(38).

Good coefficient of determination was observed between in vitro and in vivo AUC for treatment A by applying quadratic model (Table 5). While for B, it is obtained by applying cubic or quadratic models (Table 6). Such results suggest that the dissolution test of F2 mimics very closely the in vivo drug release in the GIT under the used experimental conditions.

The model equation for treatment A is:

$Y = -1E\text{-}12x^4 + 1E\text{-}08x^3 - 1E\text{-}05x^2 + 0.0049x + 0.1216$
while for B it is:
$Y = -4E\text{-}10x^3 - 3E\text{-}06x^2 + 0.0047x + 0.0945$ or
$Y = -8E\text{-}14x^4 + 1E\text{-}09x^3 - 4E\text{-}06x^2 + 0.0051x + 0.0719$
Where, Y is AUC_{0-t} in vivo
X is AUC_{0-t} in vitro

Table 5: Coefficients of determination of different regression equations correlating in vitro and in vivo AUC_{0-t} for treatment A

Regression type	Regression equation	Coefficient of determination (R^2)
Linear	$Y = 0.0007x + 0.2525$	0.9925
Logarithmic	$Y = 0.4631 \ln x - 1.0142$	0.5649
Square model	$Y = -2E\text{-}08x^2 + 0.0009x + 0.2226$	0.9931
Cubic model	$Y = -2E\text{-}10x^3 - 2E\text{-}06x^2 + 0.002x + 0.1782$	0.9951
Quadratic model	$Y = -1E\text{-}12x^4 + 1E\text{-}08x^3 - 1E\text{-}05x^2 + 0.0049x + 0.1216$	0.9975

Table 6: Coefficients of determination of different regression equations correlating in vitro and in vivo AUC_{0-t} for treatment B

Regression type	Regression equation	Coefficient of determination (R^2)
Linear	Y = 0.0011x + 0.7108	0.9861
Logarithmic	Y = 1.3043 ln x – 5.7382	0.6425
Square model	$Y = -8E\text{-}08x^2 + 0.0018x + 0.396$	0.9926
Cubic model	$Y = -4E\text{-}10x^3 - 3E\text{-}06x^2 + 0.0047x + 0.0945$	0.9999
Quadratic model	$Y = -8E\text{-}14x^4 + 1E\text{-}09x^3 - 4E\text{-}06x^2 + 0.0051x + 0.0719$	0.9999

Results of ANOVA tests for a crossover design performed to assess the effect of sequence, subjects within sequence and periods on different pharmacokinetic parameters and to compare both treatments A and B are given in Tables 7-9.

No significant difference was observed with respect to sequence, subjects within sequence and periods with $C_{max,}$ T_{max} and log AUC_{0-24}. On the other hand, a significant difference was found between treatments A and B with respect to the same previous pharmacokinetic parameters respectively.

Table 7: Analysis of variance applied to Cmax generated from crossover comparative bioavailability study data

Source	Degree of freedom	Sum of squares	Mean squares	F
Sequence	1	0.03402675	0.03402675	0.79
Subject/sequence	4	0.39352700	0.09838175	2.29
Period	1	0.01147008	0.01147008	0.27
Treatment	1	1.17125008	1.17125008	27.29**
Error	4	0.17169033	0.04292258	

*P≤0.01

Table 8: Analysis of variance applied to Tmax generated from crossover comparative bioavailability study data

Source	Degree of freedom	Sum of squares	Mean squares	F
Sequence	1	3.52083333	3.52083333	0.17
Subject/sequence	4	78.41666667	19.60416667	0.92
Period	1	38.52083333	38.52083333	1.81
Treatment	1	172.52083333	172.52083333	8.11*
Error	4	85.08333333	21.27083333	

*P≤0.05

Table 9: Analysis of variance applied to log AUC0-24 generated from crossover comparative bioavailability study data

Source	Degree of freedom	Sum of squares	Mean squares	F
Sequence	1	0.01197008	0.01197008	0.60
Subject/sequence	4	0.16353967	0.04088492	2.03
Period	1	0.00003675	0.00003675	0.00
Treatment	1	0.42225008	0.42225008	21.02**
Error	4	0.08036567	0.02009142	

*P≤0.01

CONCLUSION

From the data presented in this study, it could be concluded that there was a marked difference between colon targeted TH compressed coated tablets and tablet core formulation. This could be attributed to the successful choice of pectin as an enzyme dependent coating polymer for colon targeting. Pectin, being of natural origin, non-hygroscopic, directly compressible and cheap, is a promising excipient in the preparation of colon targeted dosage forms for oral use.

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