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Pharmacokinetic Studies Of Chronopharmaceutical, Conventional And Pure Drug Delivery System Of Theophylline By LC-MS/MS Method

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Abstract

Clinical studies have shown that circadian patterns influence the pharmacokinetics of certain drugs used in the treatment of different diseases. For such drugs, the bioavailability is influenced by the time of administration. The objective of this study was to investigate differences in the pharmacokinetic patterns between a pulsatile drug delivery system using a pulsatile capsule, pure active pharmaceutical ingredient (theophylline) and an exisisting marketed immediate release tablet (conventional). Theophylline was chosen as a model drug because of its significant solubility and permeability pattern throughout the GI tract. The dosage form of 400 mg each were administered to 3 groups of white New Zealand rabbits (n=6) following cross over design pattern and the plasma levels were measured using LC-MS/MS method. Pharmacokinetic parameters were determined for each dosage form. The comparison of the plasma time curves of the dosage forms showed that each dosage form caused significant differences in the drug plasma levels. The plasma drug profiles of active pharmaceutical ingredient and marketed conventional tablet of theophylline showed nearly similar pattern of drug release, whereas the pulsatile capsular formulation prepared in the laboratory managed to show some lag phase initially before releasing the drug. The pulsatile drug delivery capsule showed maximum time (Tmax) at the 8th hour in comaprision to active ingredient which showed plasma peak in the range of 2-3 hours. Implications for the use of a pulsatile drug delivery device for chronopharmacotherapy are discussed. Pulsatile drug delivery offers a promising way for chronopharmacotherapy if the time of administration and pulse time are adjusted to the circadian pattern.

Keywords

LC-MS/MS, Theophylline, chronopharmaceutical, In-vivo studies, New Zealand rabbits

INTRODUCTION

Chronopharmacokinetic studies have been reported for many drugs in an attempt to explain chronopharmacological phenomena and demonstrate that the time of administration is a possible factor of variation in the pharmacokinetics of a drug [1]. Chronotherapeutics is defined as a treatment system where the in vivo drug availability has been timed in accordance to cyclic rhythms of drug related biological phenomena to create maximum benefit minimizing harm [2,3]. All the current sustained release formulation have a shortcoming of inability to maintain high blood levels for that long period with high disease intensity. This may lead to leaving the patient unprotected against the worse events of nocturnal asthma[5]. Thus, a smart drug delivery that is administrated before sleep and maintains high blood levels for longer period (from midnight to 8 am in the morning, during which maximum intensity of the disease occurs) could be very much beneficial for proper management of nocturnal asthma[6-9]. Systemic absorption from the colon can also be used as a mean of achieving chronotherapy for diseases that are affected by circadian rhythms such as asthma, angina and arthritis. A successful colonic drug delivery system is one designed to protect a drug from absorption and/ or degradation during its transit through the upper gastrointestinal tract (GIT) and allow its rapid releasing in the ascending colon. Different biopharmaceutical and pharmacodynamic reasons point out that there are certain circumstances in which a repeated dosing is advantageous compared to sustained release

dosage forms. However, a patient's compliance might be lower if a drug has to be taken more than once a day[10,11]. If drug absorption throughout the gastrointestinal tract is not limited, then pulsatile drug delivery might be a suitable alternative to repeated dosing. This might be especially useful if peak plasma levels are desirable in the night time or the early morning hours. Pulsatile drug delivery system have a number of advantages like to maintain constant plasma drug level, time of administration (during morning hours), would be ideal. Same is true for preventing asthmatic attacks in the middle of the night and the morning stiffness typical of people suffering from arthritis. Drugs that produce biological tolerance demand for a system that will prevent their continuous presence at the biophase as this tends to reduce their therapeutic effect[12-15]. Clinical studies have shown that circadian patterns influence the pharmacokinetics of certain drugs used in the treatment of different diseases. For such drugs, the bioavailability is influenced by the time of administration. The objective of this study was to investigate differences in the pharmacokinetic patterns between a pulsatile drug delivery system using a pulsatile capsule, an immediate release delivery (conventional) and pure drug theophylline all of same dose[16-18]. Theophylline was chosen as a model drug because of its high solubility and high permeability pattern throughout the GI tract. The dosage forms were administered to three group's containing six of white New Zealand rabbits each, and the plasma levels were measured using LC-MS/MS. Cross-over design pattern was used for the above studies [19]. Pharmacokinetic parameters were determined for each dosage form and compared. Fluctuations in the plasma time curves over the observation period indicated that physiological factors like motility have an influence on the drug absorption. The comparison of the plasma time curves of the dosage forms showed that each dosage form caused significant differences in the drug plasma levels. The pulsatile drug delivery capsule caused two defined C(max) values for each dose between 1-1.75 and 2.5-3.5h. Implications for the use of a pulsatile drug delivery device for chronopharmacotherapy are discussed. Pulsatile drug delivery offers a promising way for chronopharmacotherapy if the time of administration and pulse time are adjusted to the circadian pattern[20-22].

MATERIALS AND METHODS

Materials

The following chemicals were obtained from commercial suppliers and used as received: Pure Theophylline (Cipla, Bangalore, India), Phenacetine-AR grade, Ethyl acetate-AR grade, Methanol-HPLC grade, Ammonium acetate-AR grade, MilliQwater-HPLC grade (S D Fine Chemicals ltd, India), Sodium Heparin , 0.45µm nylon 6,6 membrane filter, Micro tips (200µl-1000µl), Auto injection vials, RIA vials and Micro tips (200µl-1000µl).

Methods

Dosage forms

Pure theophylline , conventional marketed theophylline (400mg) and insoulble capsular pulsatile drug release of theophylline (400 mg) containing karaya gum plug prepared in the laboratory conditions and chosen on the basis of drug content , lag time achieved ,in-vitro release studies and stability conditions were chosen as dosage forms for administration.

Subjects

Eighteen New Zealand rabbits (1.5- 2.5 kg) were used in this study, chosen after Animal ethical clearance certificate from CPSCEA committee. Each group consisted of six rabbits (n=6) each and were subjected for overnight fasting, it was taken care that there was no stress on the animals. Rabbits were randomly divided into three groups for different sampling time and each group was housed in one cage. Food and water were available ad libitum at all times during the experiment. The study was conducted in a crossover design with 3 weeks washout periods in between the two experiments. The above 3 groups were labelled as A, B and C. The above dosage form was administered using sterile internal stomach pumps.

Blood sampling

Blood samples (5 ml) were collected from the tracheal lobular vein of the rabbit using and the blood was stored in screw top heparinized plastic tubes, the sampling time for blood was done at 0 mins(predose), 15 mins, 30 mins, 60 mins, 120 mins, 180 mins , 240 mins, 480 mins, 600 mins, 720 mins , 1200 mins and 1440 mins The plasma was immediately separated by aspiration after centrifugation at 4000 rpm for 5 minutes and frozen at -20 ºC until analyzed.

Preparation of Internal standard (IS) stock solution

About 2mg of internal standard (Phenacetin) was weighed accurately & transferred into a 2ml volumetric flask. It was then dissolved in Methanol and the volume was made up with the same to produce a solution of 1mg/ml strength of internal standard. The above final concentration internal standard was corrected according to its potency and actual amount weighed. It was then stored in refrigerator or cooling cabinet.

Preparation of Theophylline Standard Stock Solution

About 2mg of theophylline working standard was weighed accurately and transferred into 2ml of volumetric flask. It was then dissolved in methanol and the volume was made with the same to produce a solution of 1mg/ml strength of theophylline. The above final concentration for theophylline was corrected for accounting for its potency and the actual amount weighed. It was then stored in refrigerator or cooling cabinet.

Spiking of Plasma for Samples

0.7ml of each of the described stock dilution of Theophylline was transferred into a 10ml of volumetric flask and the volume was made up with Sodium heparin .Plasma then was pooled and mixed well.

Sample preparation

All samples of one or more periods of one or more subjects were withdrawn from the freezer or deep freezer and allowed them to thaw at room temperature. The thawed samples were vortexed to ensure complete mixing of contents. 100µl of samples were pippeted in to respectively labelled Radio-Immuno Assay (RIA) Vials. 50µl of internal standard (0.5µg/ml) were added into respectively labelled RIA vials and vortex. 0.5ml of extraction solvent (Ethyl Acetate) were added to all the RIA vials and capped. All the samples were kept in a vibramax for 10 min at 2500rpm. All the samples were centrifuged for 5min at 10000rpm in a refrigerator centrifuge. 0.4ml of organic layer was transferred into respective labelled RIA vials. The organic layer was dried in a nitrogen evaporator at 40^0C. The dried residue was reconstituted with 0.1ml of mobile phase and vortexed. Reconstituted samples were transferred in to respectively labelled auto injection vials. 5µl of the above was then injected in to LCMS/MS system using the chromatographic condition described below.

Chromatographic Conditions

Column composed of hypurity advance C18 Column (3x50mm), Mobile Phase composed of 2mM Ammonium acetate: Methanol:: 20:80V/V (Binary Flow) mixture, the injection volume was about 5µl, the Flow rate was about 0.2ml/min without splitter (Binary Flow), and the Run time was fixed at 3 minutes, the Column oven temperature was about 40^0C and the sample cooler temperature was fixed at 10^0C.

Calculation of the concentration

The concentration of the unknown was calculated from the following method using regression analysis of spiked plasma calibration standard with the reciprocal of the square of the drug concentration as weighing factor (1/concentration X concentration)

$$y = mx + b$$

Where

X – Concentration Analyte

m – Slope of the calibration curve

Y – Peak area ratio of analyte to internal standard (IS)

b – Y-axis intercept of the calibration curve

Data Analysis

Pharmacokinetic parameters were estimated using model-independent methods (Gibaldi and Perrier, 1982). WINNOLIN Scientific Software, Statistical Consultant, Apex, NC, USA), nonlinear least squares regression, computer programs, was utilized to estimate the pharmacokinetic parameters of

theophylline. The noncompartmental analysis for extravascular administration in WINNOLIN was used to measure the area under theophylline concentration time curve (AUC) for a period of 1440 minutes (t= 1440 minutes), the area under the first moment of the curve (AUMC = *JtCdt),* the mean residence time (MRT = AUMC/AUC). The apparent total clearance *(Cl/F)* was calculated using noncompartmental equations where, CI/ i^7 = (dose/AUC). The Annova software was used to determine statistically significant differences (P<0.05) of in vivo data.

Statistical analysis

One way analysis of variance (ANOVA) using Dunnett multiple comparison test on computer program Graphpad Instat 3 was used.

RESULTS AND DISCUSSION

In vivo bioavailability studies are important to establish recommended dosage regimens and to support drug labeling. This can be achieved by determination of the essential pharmacokinetic parameters which include the rate and extent of systemic absorption, elimination half life, and rate of execration and metabolism. Bioavailability is considered as one aspect of drug product quality that link in-vivo performance of a drug product used in clinical trials to studies demonstrating evidence of safety and efficacy. The primary objective for developing any dosage formulation is to deliver the required concentration of an active drug substance to the site of action and to achieve optimum efficacy by LCMS/MS method. Various chromatograms for plasma drug concetrations as shown in Figure no 1 were obtained. Fragmented mass of all the dosage forms as shown in Figure no 2 were obtained and studied.

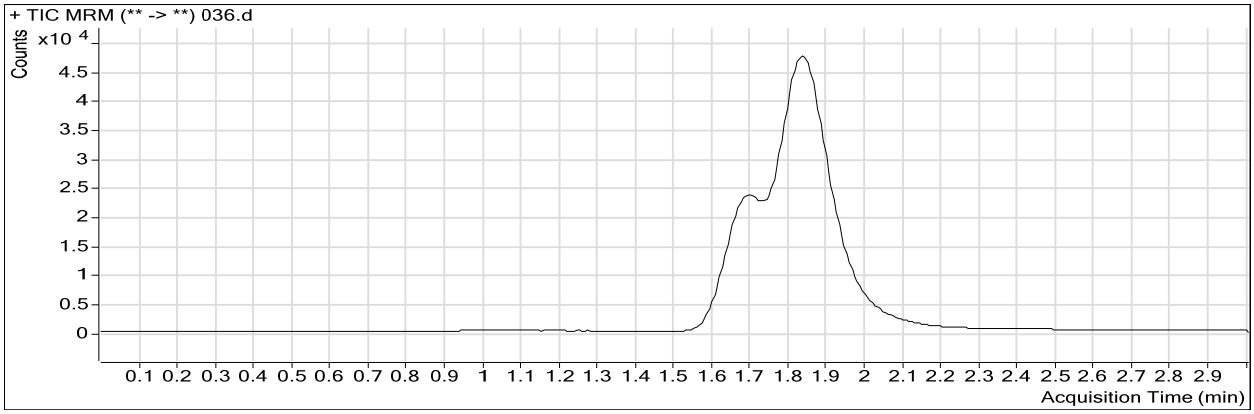

Figure no 1 : Chromatogram of plasma sample for pulsatile drug delivery

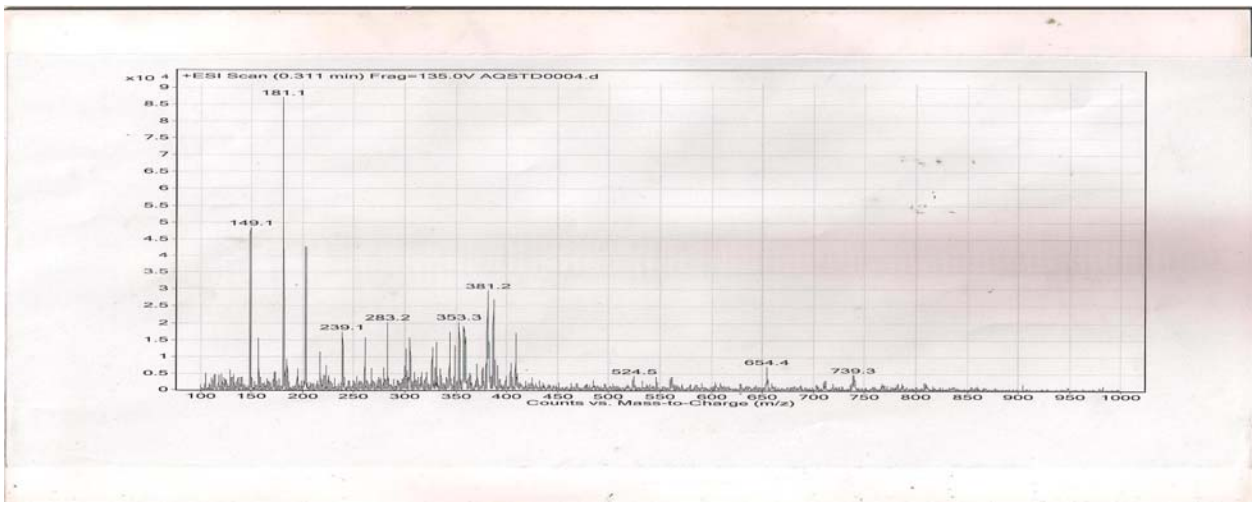

Figure no 2: Fragmented mass spectra of plasma drug sample of theophylline as obtained by LCMS/MS

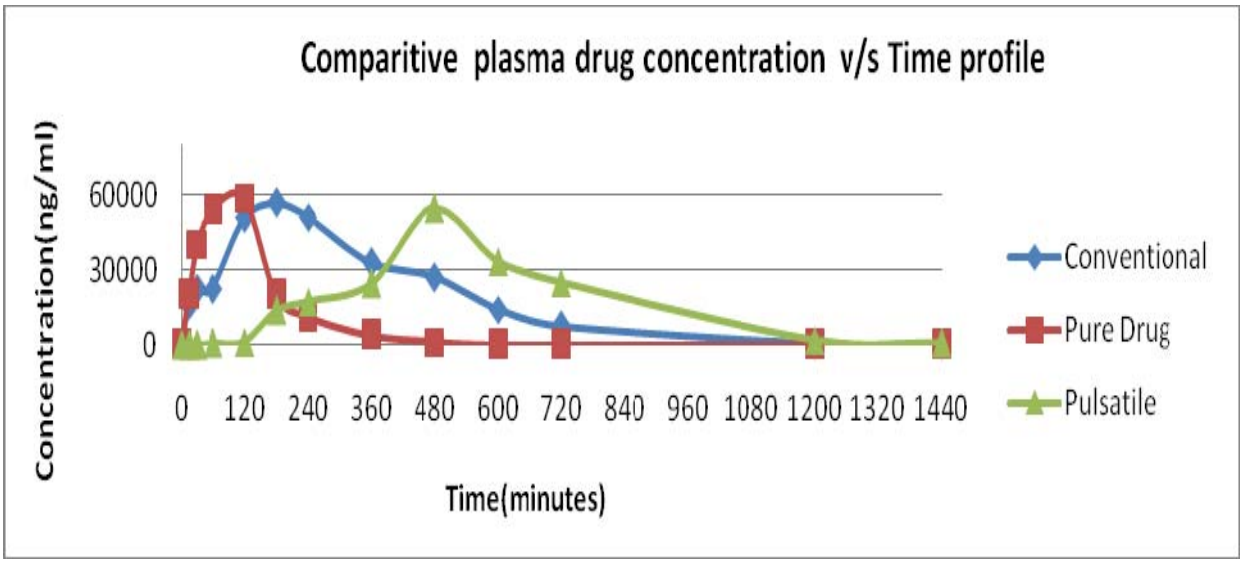

Figure no 3: Plasma-drug concentration v/s time graph of all the dosage forms as measure by LC-MS/MS method

The ability of pulsatile capsules as a drug delivery system to release drugs in a pre-determined time release manner was investigated in New Zealand rabbits after oral administrations was investigated. Theophylline was used as the marker drug. The pulsatile drug delivery system prepared under laboratory conditions release the drug in –vitro in a uniform and reliable manner, these data indicated that the device should be suitable for in-vivo evaluation in animals. Mean plasma drug concentration curve v/s time as shown in Figure no 3 of all the groups of rabbits was studied for studying and comparing various pharmacokinetic parameters. Maximum drug plasma concentration (C max) and the time to maximum value (T max) were obtained directly from the drug plasma profile for each animal following administration of all the three above mentioned dosage formulations.

The AUC 0-24 for animals (Group A given pure drug theophylline was found to be 19863219.9 nanograms/ml/hr and animals given marketed conventional tablet of theophylline (400 mg), AUC 0-24 was found to be 23885079.86 nanograms/ml/hr whereas the AUC 0-24 for animals administered with pulsatile release AUC 0-24 was found to be 24379126.35 nanograms/ml/hr. MRT is defined as the mean time for the intact drag molecule to transit through the body and involved a composite of all kinetic processes including release from the dosage form, drug absorption into the body and drug disposition. MRT can be used in a comparative way to evaluate the in vivo performance of a pulsatile release dosage form. Therefore, the increase in the MRT from 2.014 to 8 hours following theophylline pure drug and pulsatile drug, respectively, was mainly due to the change in drug release and elimination. The average t_{max} values were found to be 3 ± 0.12hr (180 mins), 2.0 ± 0.78 hr (120 mins), and 8.0 ± 0.95hr (480 mins) for marketed conventional, pure drug theophylline and pulsatile drug respectively. Pure drug formulation showed low value of t_{max} (2 hours) which indicates faster absorption of the drug as compared to pulsatile drug formulation. As per the summary of pharmacokinetic parameters as given in Table no 1 one can predict that pure drug theophylline and marketed conventional formulation showed almost similar pattern of drug absorption and pulsatile drug formulation showed a lag time of 3 hours before finally showing maximum concentration (Cmax) at 8 hours, which correlated with the in-vitro release (8 hours). One way analysis of variance (ANOVA) using Dunnett multiple comparison test on computer program Graphpad Instat 3 was used, the differences were considered significant at p value equal or less than 0.05 ($p \leq 0.05$).

Pharmacokinetic Parameters	*Group A (Marketed Conventional)*	*Group B (Pure drug)*	*Group C (Pulsatile)*
AUC 0-24 (nanograms/ml/hr)	23885079.86 ± 1.24	19863219.9 ± 0.84	24379126.35 ± 2.35
(AUC) t-∞ (nanograms/ml/hr)	23885130.67 ± 0.46	19863332.2 ± 0.55	24379234.45 ± 0.87
AUMC 0-24 (nanograms/ml/hr^2)	118609396537 ± 0.23	1194385860 ± 1.85	13481239890.6 ± 0.86
Cmax, ng/ ml	56699.516 ± 0.81	58734.612 ± 0.34	54632.231 ± 0.43
tmax,hr	3 ± 0.12hr (180 mins)	2.0 ± 0.78 hr (120 mins)	8.0 ± 0.95hr (480 mins)
$t_{1/2, hr}$	2.3 ± 0.78 hours	1.9 hours	7.8 ± 0.45hours
Kel(hr^{-1})	0.11 ± 0.65	0.14 ± 1.83	0.21 ± 0.55
MRT(hrs)	2.9 ± 3.33 hours	2.014 ± 2.34hours	9.2 ± 4.85hours

Table no 1: **Summary of the pharmacokinetic parameters of all the groups of rabbits**

CONCLUSION

The developed LC-MS/MS method was highly sensitive and suitable for the detection of theophylline in plasma in concentrations as low as 0.5 nanogram/ ml. In conclusion, pulsatile drug release over a period of 4-12 hrs, consistent with requirements for chronopharmaceutical drug delivery, was achieved from a insoluble gelatin capsule where drug was sealed within the capsule body by means of an erodible plug. Thus, classical pulsatile capsular formulation parameters could be manipulated to modulate the drug release time in accordance with chronotherapeutic objectives.

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