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Pharmacokinetic studies on pulsatile drug delivery of natural non steroidal anti inflammatory drug: LC-MS/MS method

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Abstract

The goal of this study was to explore variations in the pharmacokinetic patterns among a pulsatile drug delivery system using a pulsatile dosage forms (capsule and tablets), pure active pharmaceutical ingredient (curcumin) and an existing marketed immediate release formulation (conventional). The dosage form of 450 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 formulation of curcumin showed nearly similar pattern of drug release, whereas the pulsatile drug delivery systems showed a lag time of near about 4 hours before finally showing maximum concentration (Cmax) at near about12 hours, which correlated with the in-vitro release (12 hours).

Keywords: LC-MS/MS, Curcumin, In-vivo studies, New Zealand rabbits

Introduction

Pulsatile drug delivery systems have a number of advantages like to maintain constant plasma drug level, time of administration, would be ideal. 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[1]. 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 and tablets, an immediate release delivery (conventional) and pure drug curcumin all of same dose[2]. Curcumin was chosen as a model drug because it is used in inflammatory bowel disease. 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 plasma time curves of the dosage forms showed that each dosage form caused significant differences in the drug plasma levels.

Material and methods

The study was carried out only on the selected formulations as the pulsatile capsule (3% as a first coat and 5 % as a second coat), sodium alginate – HPMC based colon targeted tablet (compressed coated-SC2) and sesbania gum based colon targeted tablet (compressed coated-CS2) as group C, D and E respectively. Consider pure drug and conventional marketed capsule of curcumin as a group A and B respectively. Thirty 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 5 groups were labeled as A, B, C, D and E. The above dosage form was administered using sterile internal stomach pumps[3]. ***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, 60 mins, 120 mins, 180 mins, 300 mins , 480 mins, 600 mins, 720 mins, 1080 mins, 1200 mins, 1320 min 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[4]. ***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[5]. ***Preparation of curcumin standard stock solution:*** About 2mg of curcumin 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 curcumin. The above final concentration for curcumin was corrected for accounting for its potency and the actual amount weighed. It was then stored in refrigerator or cooling cabinet[6]. ***Spiking of plasma for samples:*** 0.7ml of each of the described stock dilution of curcumin 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 labeled Radio- Immuno Assay (RIA) Vials. 50µl of internal standard (0.5µg/ml) were added into respectively labeled 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 labeled RIA vials. The organic layer was dried in a nitrogen evaporator at 400C. The dried residue was reconstituted with 0.1 ml of mobile phase and vortexes. Reconstituted samples were transferred in to respectively labeled auto injection vials. 5µl of the above was then injected in to LCMS/MS system using the chromatographic condition described below[7]. ***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

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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 400C and the sample cooler temperature was fixed at 100^0C[7]. ***Calculation of the concentration***[8]***:*** The concentration of the unknown was calculated 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). ***Data analysis:*** Pharmacokinetic parameters were estimated using model-independent methods (Gibaldiand Perrier, 1982). WINNOLIN Scientific Software, Statistical Consultant, and Apex, NC, USA), nonlinear least squares regression, computer programs, was utilized to estimate the pharmacokinetic parameters of curcumin. The no compartmental analysis for extra vascular administration in WINNOLIN was used to measure the area under curcumin 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 no compartmental equations where, CI/ i[7] = (dose/AUC). The Annova software was used to determine statistically significant differences (P<0.05) of in vivo data[9].

Results and Conclusion

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 concentrations as shown in Figure 1 were obtained. Fragmented mass of all the dosage forms as shown in Figure 2 were obtained and studied. The ability of pulsatile capsule and tablets as a drug delivery system to release drugs in a predetermined time release manner was investigated in New Zealand rabbits after oral administrations was investigated. Curcumin was used as the marker drug. The pulsatile drug delivery system prepared under laboratory conditions released 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 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 curcumin was found to be 19863219.9 nanograms/ml/hr and animals given marketed conventional capsule of curcumin (450 mg), AUC 0-24 was found to be 23885079.86 nanograms/ml/hr whereas the AUC 0-24 for animals administered with pulsatile release capsule AUC 0- 24 was found to be 24379126.35 nanograms/ml/hr, pulsatile release tablet of SC2 AUC 0-24 was 24369125.45 and pulsatile release tablet of CS2 AUC 0-24 was 24259126.35. 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 forms. Therefore, the increase in the MRT from 2.014 to near about 12 hours following curcumin pure drug and pulsatile drug delivery systems, 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), 12.0 ± 0.95hr (720 mins), 11.9 ± 0.95hr (720 mins) and 12.02 ± 0.90hr (720 mins) for marketed conventional, pure drug curcumin, pulsatile capsule, pulsatile tablet of SC2 and pulsatile tablet of CS2 respectively. Pure drug formulation showed low value of tmax (2 hours) which indicates faster absorption of the drug as compared to pulsatile drug delivery systems. As per the summary of pharmacokinetic parameters as given in Table 1 one can predict that pure drug of curcumin and marketed conventional formulation showed almost similar pattern of drug absorption and pulsatile drug delivery systems showed a lag time of near about 4 hours before finally showing maximum concentration (Cmax) at near about12 hours, which correlated with the in-vitro release (12 hours). One way analysis of variance (ANOVA) using Dunnett multiple comparison test on computer program Graph pad Instant 3 was used, the differences were considered significant at p value equal or less than 0.05 ($p \leq 0.05$).

The developed LC-MS/MS method was highly sensitive and suitable for the detection of curcumin in plasma in concentrations as low as 0.5 nanogram/ ml. In conclusion, pulsatile drug release over a period of 5-12 hrs, consistent with requirements for colon targeted drug delivery, was achieved from a modified released capsule and tablets. Thus, classical pulsatile formulations parameters could be manipulated to modulate the drug release time in accordance with research objectives.

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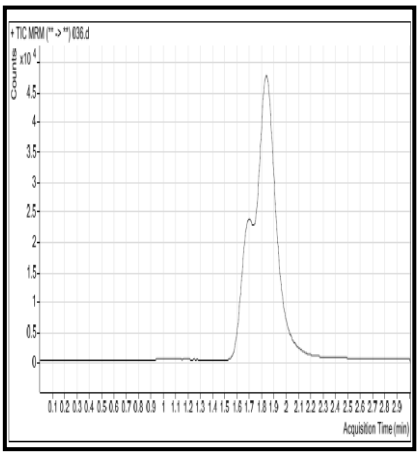
Table 1: Summary of the pharmacokinetic parameters of all the groups of rabbits

Pharmacokinetic Parameters	Group A (Marketed Conventional)	Group B (Pure drug)	Pulsatile dosage forms		
			Group C (Capsule 3%-5%)	Group D (Tablet SC2)	Group E (Tablet CS2)
AUC 0-24 (nanograms/ml/hr)	23885079.86 ± 1.24	19863219.9 ± 0.84	24379126.35 ± 2.35	24369125.45 ±2.25	24259126.35 ±2.32
(AUC) t-∞ (nanograms/ml/hr)	23885130.67 ± 0.46	19863332.2 ± 0.55	24379234.45 ± 0.87	24369226.55 ±0.85	24259236.45 ±0.84
AUMC 0-24 (nanograms/ml/hr2)	118609396537 ± 0.23	1194385860 ± 1.85	13481239890.6 ± 0.86	13370239891.5 ±0.87	13282238889.7 ±0.85
Cmax, ng/ ml	56699.516± 0.81	58734.612 ± 0.34	54632.231 ± 0.43	54521.122 ± 0.44	54887.342 ± 0.45
tmax,hr	3 ± 0.12hr (180 mins)	2.0 ± 0.78 hr (120 mins)	12.0 ± 0.95hr (720 mins)	11.99 ± 0.95hr (720 mins)	12.02 ± 0.90hr (720 mins)
t 1/2, hr	2.3 ± 0.78 hr	1.9 hr ± 0.78 hr	11.8 ± 0.45hr	11.9 ± 0.44hr	11.7 ± 0.49hr
Kel(hr-1)	0.11 ± 0.65	0.14 ± 1.83	0.21 ± 0.55	0.22 ± 0.50	0.20 ± 0.54
MRT(hrs)	2.9 ± 3.33 hrs	2.014 ± 2.34 hrs	13.2 ± 4.85hrs	13.1 ± 4.88hrs	13.0 ± 4.90hrs



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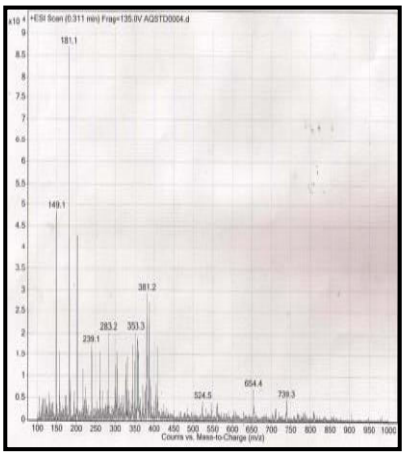

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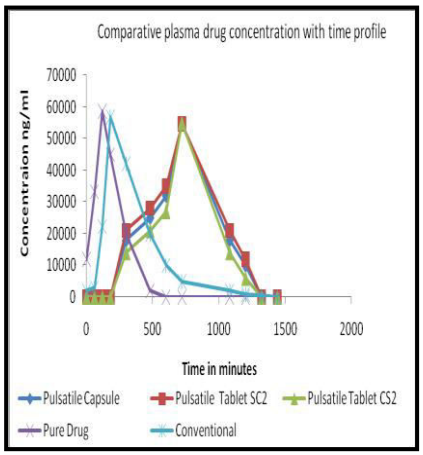

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Fig. 1: Chromatogram of plasma sample for pulsatile drug delivery
Fig. 2: Fragmented mass spectra of plasma drug sample of curcumin
Fig. 3: Comparative plasma-drug concentrations of all the dosage forms