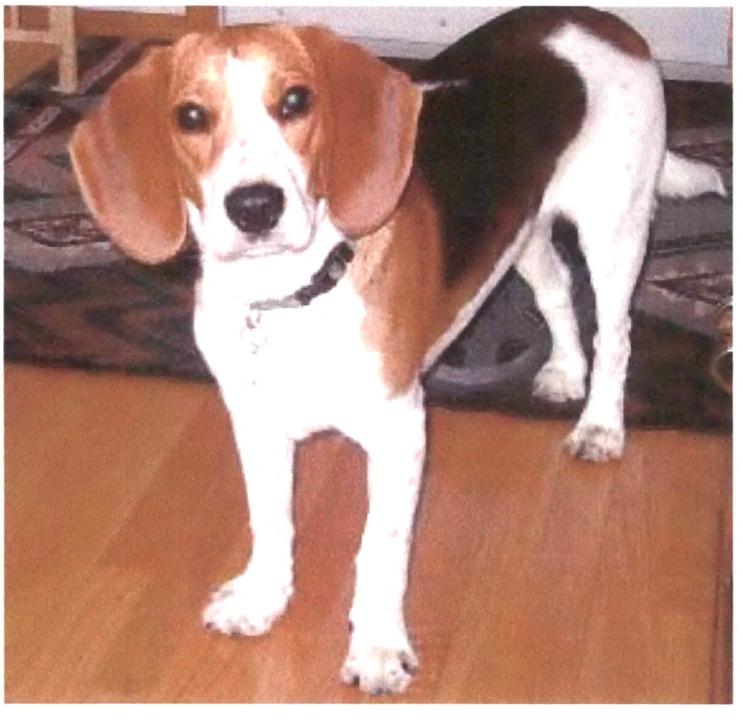
CHAPTER 4



IN-VIVO STUDIES

IN-VIVO PHARMACOKINETIC STUDY DESIGN & PROTOCOL -GLIPIZIDE

Introduction:

In -vivo evaluation of glipizide SPOP tablets and Glucotrol XL tablets were performed in six beagle dogs using a two-way comparative cross-over design in accordance with GLP compliance for conducting pharmacokinetics studies. The protocol for general procedures and use of animals for conducting the said study has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC). CPCSEA approved protocol : ZRC/CPCSEA/DMPK/164a(e)/03-2K7.

***Test Animals*:**

The canines were supplied by Animal House of Cadila Healthcare Ltd. registered under Rule 5 (a) of the "Breeding of and Experiments on Animals (Control and Supervision) Rules 1998" (Registration No. 77 / 1999 / CPCSEA). The study was performed at Canine Research Facility and Drug Metabolism and Pharmacokinetics Laboratory of Zydus Research Centre, Ahmedabad.

Animals were acclimatized for at least 1 week prior to the study in an environmentally controlled room (in compliance with the guidelines provided by CPCSEA, Govt of India) with 25 ± 5 ^{0}C temperature and 30-70% humidity.

The dogs were approximately 9 to 14 kg in weight and 1 year old. Each canine had individual kennel number and an ear tattoo (performed by breeder) for identification and was housed individually in kennel. Each cage had an identification card showing the study number, canine number, species, strain and sex.

Dosage Forms Administered, Frequency and Method of Dosing

The *in-vivo* evaluation of glipizide SPOP tablets (10-mg, Lot No. GZ-10/F09) was tested against Glucotrol XL tablets (10-mg, Lot No. 4XP046E) manufactured by Pfizer Inc. All the test articles were stored in a locked area at ambient temperature. The canines were fed with standard laboratory animal diet eight hours after dosing. Purified water was available *ad libitum* throught the experiment. This water monitored for bacterial contamination periodically as per the SOP.

No contaminants expected to interfere with the study were known to be present in the feed or water. Each dog received one 10-mg Glipizide SPOP tablets or 10-mg Glucotrol

XL tablets in fasted state. Following a 1-week washout period, each dog received a different formulation in Phase II. The experimental protocol details are provided in Table 4.1.

Table 4.1 In-vivo evaluation study protocol details

Dosage form	Condition	Dose (mg/dog)	No. of tablets
Phase I			
Glipizide SPOP tablets	Fasted	10	1
One week wash out period			
Phase II			
Glucotrol XL tablets	Fasted	10	1

Blood Sampling

Blood samples (5 mL) were taken from each dog at 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours after dosing for both the formulations. The samples collected were transferred into test tubes containing heparin saline solution (used as an anticoagulant), and to prevent decomposition, they were placed in an ice bucket prior to centrifugation. Plasma was separated following cold centrifugation and was frozen in amber glass vials at -20°C before analysis.

Processing Blood Samples for HPLC

0.5 ml of plasma was acidified with 200µl 0.5 M HCl and vortex-mixed 1 min. Acidified plasma was vortex-mixed with 3 ml benzene for 5 min and centrifuged at 3500 rpm for 10 min. The organic layer was dried at 40°C under a stream of nitrogen and the residue was dissolved in 100 µl methanol. Briefly, 20 µl of the filtrate was injected into HPLC system. (Defang et al 2005)

Chromatographic Conditions

Column	: RP C18, (25 cm x 4.6 mm), 5 μm
Mobile Phase	: Acetonitril : Methanol : Water (35:10:55)
Detector (UV)	: 225 nm
Flow rate	: 1 mL/min.
Injection volume	: 10 μL
Column temperature	: 40°C

Calibration Graph

The calibration graph of glipizide was linear between 20 and 600 ng/ml and the correlation coefficient of the calibration graph was 0.9918. The detection limit was 8 ng/ml. The recovery of the 20, 100 and 600 ng/ml of glipizide was 98.1 ± 0.9%, 99.1 ± 0.6% and 99.2 ± 0.4%, respectively. The precision of the 20, 100 and 600 ng/ml of glipizide was 0.88%, 0.91% and 0.86%, respectively.

Pharmacokinetic Analysis

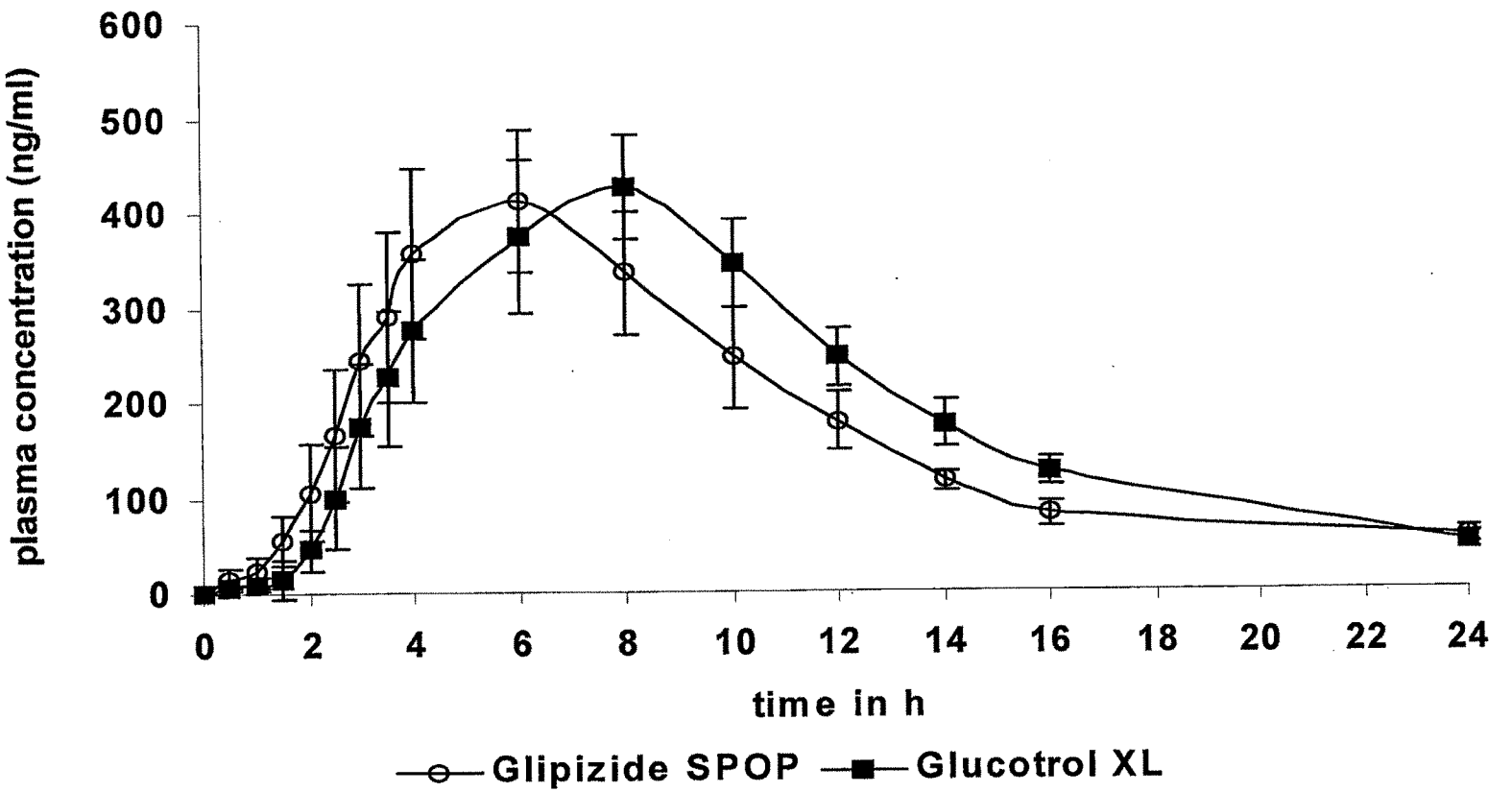
The most suitable model to describe the pharmacokinetics of glipizide was determined by fitting the data to a hierarchy of models using WinNonlin software version 5.0.1 (Pharsight Corporation, USA). The data most appropriately fitted to a noncompartmental model and pharmacokinetic parameters, such as C_{max}, T_{max} and $AUC_{0\text{-}24\ h}$ were calculated by a computer using the same WinNonlin software.

Results and Discussion:

Table 4.2 shows the mean pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{0\text{-}24\ h}$) determined for both dosage forms. Figure 4.1 shows the glipizide plasma concentration profile for 24 hours following administration of both dosage forms.

The mean T_{max} for glipizide SPOP tablets shown in Table 4.2 was 6.11 hours compared to 8.95 hours for the Glucotrol XL tablets. This indicated that the time taken to reach maximum plasma glipizide concentrations were comparable in both the formulations, thus providing controlled release of the drug. The mean $AUC_{0\text{-}24\ h}$ of the glipizide SPOP was 3648.49 ng.h/ml as compared to 4155.76 ng.h/ml that of Glucotrol XL. Also the C

Fig 4.1. Comparison of In-Vivo Pharmacokinetic Profile of Glipizide SPOP Tablets and Glucotrol XL Tablets in Beagle Dogs.



$_{max}$ of the glipizide SPOP was 411.97 ng/ml as compared to 425.27 ng/ml that of Glucotrol XL. However the T/R ratio was within the limit of 80-125%, taking the values of glipizide SPOP as T and the value of Glucotrol XL as R, suggesting that the developed glipizide SPOP tablets were bioequivalent with Glucotrol XL.

Table 4.2 Mean Pharmacokinetics Parameters of Glipizide SPOP tablets and Glucotrol XL tablets obtained by Noncomparmental Analysis on six Beagle Dogs.

Dosage form	C_{Max} (ng/ml)	T_{Max} (h)	$AUC_{0-24\ h}$ (ng h/ml)
Glipizide SPOP tablets	411.97	6.11	3648.49
Glucotrol XL tablets	425.27	8.95	4155.76
T/R %	96.87	-	87.79

IN-VIVO PHARMACOKINETIC STUDY DESIGN & PROTOCOL -NIFEDIPINE

Introduction:

In -vivo evaluation of nifedipine MOTS tablets and Procardia XL tablets were performed in six beagle dogs using a two-way comparative cross-over design in accordance with GLP compliance for conducting pharmacokinetics studies. The protocol for general procedures and use of animals for conducting the said study has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC). CPCSEA approved protocol: ZRC/CPCSEA/DMPK/164a(e)/03-2K7.

***Test Animals*:**

The canines were supplied by Animal House of Cadila Healthcare Ltd. registered under Rule 5 (a) of the "Breeding of and Experiments on Animals (Control and Supervision) Rules 1998" (Registration No. 77 / 1999 / CPCSEA). The study was performed at Canine Research Facility and Drug Metabolism and Pharmacokinetics Laboratory of Zydus Research Centre, Ahmedabad.

Animals were acclimatized for at least 1 week prior to the study in an environmentally controlled room (in compliance with the guidelines provided by CPCSEA, Govt of India) with 25 ± 5 ^{0}C temperature and 30-70% humidity.

The dogs were approximately 9 to 14 kg in weight and 1 year old. Each canine had individual kennel number and an ear tattoo (performed by breeder) for identification and was housed individually in kennel. Each cage had an identification card showing the study number, canine number, species, strain and sex.

Dosage Forms Administered, Frequency and Method of Dosing

The *in-vivo* evaluation of nifedipine MOTS tablets (30-mg, Lot No. NP-30/F08) was tested against Procardia XL tablets (30-mg, Lot No. 05-4468-32-7) manufactured by Pfizer Inc. All the test articles were stored in a locked area at ambient temperature. The canines were fed with standard laboratory animal diet eight hours after dosing. Purified water was available *ad libitum* throught the experiment. This water monitored for bacterial contamination periodically as per the SOP.

No contaminants expected to interfere with the study were known to be present in the feed or water. Each dog received one 30-mg nifedipine MOTS tablets or 30-mg Procardia

XL tablets in fasted state. Following a 1-week washout period, each dog received a different formulation in Phase II. The experimental protocol details are provided in Table 4.3.

Table 4.3 In-vivo evaluation study protocol details

Dosage form	Condition	Dose (mg/dog)	No. of tablets
Phase I			
Nifedipine MOTS tablets	Fasted	30	1
One week wash out period			
Phase II			
Procardia XL tablets	Fasted	30	1

Blood Sampling

Blood samples (5 mL) were taken from each dog at 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours after dosing for both the formulations. The samples collected were transferred into test tubes containing heparin saline solution (used as an anticoagulant), and to prevent decomposition, they were placed in an ice bucket prior to centrifugation. Plasma was separated following cold centrifugation and was frozen in amber glass vials at -20°C before analysis.

Processing Blood Samples for HPLC

Methanol (100 mL) containing 2 mg/mL butamben, used as an internal standard, and acetonitrile (2 mL) were added to 0.5 mL of plasma in a test tube and were agitated in a vortex mixer for 30 minutes. After centrifugation at 4,000 rpm for 20 minutes, 2 mL of the supernatant was transferred into a test tube containing 1 mL of distilled water. To this solution, 4.5 mL of acetone-chloroform mixture (1:1 v/v) was added. This mixture was then agitated for 1 hour on a vortex mixture to ensure complete extraction of nifedipine into the organic phase and was then centrifuged at 4,000 rpm for 20 minutes to separate the organic and aqueous phases. The aqueous phase was discarded, and 5 mL of the

organic phase was transferred to a fresh test tube and was reduced to dryness in a sample concentrator under nitrogen at 45° C for 30 minutes. The residue was dissolved in 100 mL of the mobile phase, and 20 µl of the solution was injected into the HPLC system. (Mehta et al.)

Chromatographic Conditions

Column	: RP C18, (25 cm x 4.6 mm), 5 µm
Mobile Phase (45:55)	: 0.01 M disodium hydrogen phosphate buffer-methanol
Detector (UV)	: 237 nm
Flow rate	: 0.8 mL/min.
Injection volume	: 10 µL
Column temperature	: 40°C

Calibration Graph

Standard solutions containing 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 10.0 mg/mL nifedipine in methanol that contained 2 mg/mL butamben (internal standard) were prepared under yellow light. The standard solution (100 mL) was added to 0.5 mL of drug-free plasma, and the samples were processed as described above. The ratios of the peak height of nifedipine to that of butamben were used to construct a calibration graph. Stock solutions of both nifedipine and the internal standard (1 mg/mL in methanol) were stored in complete darkness. These solutions were freshly prepared every 2 weeks. Precision obtained using the described technique was +5%.

Pharmacokinetic Analysis

The most suitable model to describe the pharmacokinetics of glipizide was determined by fitting the data to a hierarchy of models using WinNonlin software version 5.0.1 (Pharsight Corporation, USA). The data most appropriately fitted to a noncompartmental model and pharmacokinetic parameters, such as C_{max}, T_{max} and $AUC_{0\text{-}24\ h}$ were calculated by a computer using the same WinNonlin software.

Results and Discussion:

Table 4.4 shows the mean pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{0\text{-}24\ h}$) determined for both dosage forms. Figure 4.2 shows the niedipine plasma concentration profile for 24 hours following administration of both dosage forms.

The mean T_{max} for nifedipine MOTS tablets shown in Table 4.4 was 11.68 hours compared to 12.05 hours for the Procardia XL tablets. This indicated that the time taken to reach maximum plasma nifedipine concentrations were comparable in both the formulations, thus providing controlled release of the drug. The mean $AUC_{0\text{-}24\ h}$ of the nifedipine MOTS was 5439.24 ng.h/ml as compared to 6084.45 ng.h/ml that of Procardia XL. Also the C_{max} of the nifedipine MOTS was 325.47 ng/ml as compared to 361.76 ng/ml that of Procardia XL. However the T/R ratio was within the limit of 80-125%, taking the values of nifedipine MOTS as T and the value of Procardia XL as R, suggesting that the developed nifedipine MOTS tablets were bioequivalent with Procardia XL.

Table 4.4 Mean Pharmacokinetics Parameters of Nifedipine MOTS tablets and Procardia XL tablets obtained by Noncomparmental Analysis on six Beagle Dogs.

Dosage form	C_{Max} (ng/ml)	T_{Max} (h)	$AUC_{0\text{-}24\ h}$ (ng h/ml)
Nifedipine MOTS tablets (T)	325.47	12.05	5439.24
Procardia XL tablets (R)	361.76	11.68	6084.45
T/R %	89.96	-	89.39

Fig 4.2. Comparison of In-Vivo Pharmacokinetic Profile of Nifedipine MOTS Tablets and Procardia XL Tablets in Beagle Dogs.