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PHYSICAL, CHEMICAL, AND BIOAVAILABILITY STUDIES OF PARENTERAL DIAZEPAM FORMULATIONS CONTAINING PROPYLENE GLYCOL AND POLYETHYLENE GLYCOL 400

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ABSTRACT

Parenteral diazepam formulations (10 mg/ml), XXV and XXX were prepared using propylene glycol and/or polyethylene glycol 400. The viscosity of formulations XXV and XXX was found to be 9.72 and 11.13 Cps., respectively using Stormer viscosimeter at 22° C. The accelerated stability studies indicated shelf-life of formulations XXV and XXX to be 132

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and 171 days, respectively. The bioavailability of these formulations was determined after intramuscular injection of 2 mg/kg diazepam into the dog using Valium injection as a reference treatment. The peak plasma diazepam concentration (C_{max}) and time to peak (t_{max}) were not significantly different for all formulations ($p>0.05$). Relative bioavailability for formulations XXV and XXX was 1.03 ± 0.33 and 1.21 ± 0.14, respectively compared to the Valium injection. The higher viscosity of formulations XXV and XXX did not alter the bioavailability of diazepam in the dog.

INTRODUCTION

Diazepam, a 1,4-benzodiazepine derivative has been commonly used as a sedative and hypnotic agent (1, 2). Valium is a commercial preparation of diazepam manufactured by Hoffman La Roche. Diazepam is lipid soluble and relatively water insoluble. The limited water solubility of diazepam necessitates its formulation into a solution containing propylene glycol (PG) (40%) and ethanol (10%), buffered with sodium benzoate and benzoic acid (5%), and preserved with benzyl alcohol (1.5%) (3).

Intravenous administration of Valium has been shown to result in pain at the site of injection and venous complications (4-6). The cause of these adverse reactions has been implicated to both diazepam and its solvent PG (7-9). In animal experiments adverse reactions of PG have been demonstrated (10-13). Cremophore and fat emulsion have

been used as alternative solvents to PG for parenteral diazepam. The pain at the site of injection and adverse venous reactions have been reduced with these solvents (14, 15), however, there are some reports of anaphylactic reactions associated with diazepam in Cremophore (14, 16). In humans, intramuscular absorption of diazepam has been reported to be slower and more variable compared to the oral administration (17). Intramuscular injection of diazepam has also been demonstrated to cause local muscle injury in animals and probably in man (18). The solvent PG was suggested to be responsible for the muscle destruction, pain observed after IM injection, and poor absorption of diazepam from the muscle (19).

In the present investigation, we prepared parenteral diazepam formulations by either reducing or replacing PG contained in Valium by polyethylene glycol 400 (PEG 400). The selected formulations were evaluated for viscosity and stability determinations. The bioavailability of diazepam from these formulations was studied in dog after a single IM injection. Valium injection was used as a reference treatment for the bioavailability study.

MATERIALS AND METHODS

Materials

Diazepam and flunitrazepam were obtained from Hoffman La Roche (Brampton, Ontario). Propylene glycol was obtained from Allen and Hanburys (Toronto, Canada) and all

other chemicals were reagent or HPLC grades (Fisher Scientific Co., Nepean, Ottawa). The following clinical supplies were used: Sure-Sep II serum plasma separator (General Diagnostics, NJ, USA), catheter (Critikon Canada Inc., Markham, Ontario), heparin male slip adapter (Medex Inc., Millard, Ohio, USA), needles 21 g, vacutainers, and syringes (Becton Dickinson & Co., Missisauga, Ontario), heparin (RIES Biologicals Inc., Irvine, CA), sodium chloride injection, USP (Baxter Travenol Laboratories of Canada Ltd., Malton, Ontario). For animals, mongrel dogs (13.0 ± 1.1 kg) from the Central Animal Care Services (University of Manitoba, Winnipeg, Canada) were used.

Formulation of Diazepam

Propylene glycol, PEG 400, 95% ethanol and benzyl alcohol were mixed in various proportions using a magnetic stirrer in an Erlenmeyer flask. Diazepam 10 mg/ml was dissolved with stirring in this nonaqueous solvent. Citrate buffer (0.5M) pH 3.5 was added slowly with constant stirring until the final volume was made up to 100 ml. The pH of the final solution was 4.5. This solution was filtered using 0.45-μ filter units (Millipore Corporation, Bedford, MA, USA) under vacuum. The composition of formulations is shown in Table 1.

Viscosity Determination

The viscosity of the selected diazepam formulations was determined using a Stormer viscosimeter (Arthur H. Thomas Co., Philadelphia, USA). The Stormer viscosimeter was calibrated using glycerine and water solutions containing 0, 10,

TABLE 1

Composition of Diazepam Formulations

Formula	% Composition		
	Valium	XXV	XXX
Diazepam	0.5	1.0	1.0
Propylene glycol	40.0	15.8	---
Polyethylene glycol 400	---	35.0	50.8
Ethanol (95%)	10.0	17.6	17.6
Benzyl alcohol	1.5	1.6	1.6
Benzoic acid	0.12	---	---
Sodium benzoate	4.88	---	---
Citrate buffer (0.5M)	---	4.0	4.0
Water ad to make	100	100	100

20, 30, 40, 50, 60, 70, 80, 90, and 100% glycerine. The viscosity determinations were repeated five times for each formulation.

Stability Studies

One ml samples of formulations XXV and XXX were placed in 2 ml glass ampoules and the ampoules were sealed (Cozzoli Machine Co., Plainfield, NJ, USA). The samples were kept in waterbaths (Blue M Electric Co., Blue Island, Illinois, USA) maintained at 95±1, 90±1, 85±1, and 80±1° C. The samples

were removed at suitable time intervals over a period of 30 days and allowed to cool to room temperature and analyzed for diazepam content using a gas chromatographic procedure (20).

Gas Chromatography Method for the Analysis of Diazepam

In brief, 0.5 ml aliquot of diazepam samples were pipetted into 15 ml screw-top test tubes. To these samples 200 µl mepyramine maleate solution (5 mg/ml) as internal standard, 2 ml ammonium hydroxide solution (0.88 g/ml), 10 ml ether, and approximately 2 g of ammonium sulfate were added. The mixtures were shaken for 10 minutes and centrifuged for 5 minutes. The organic phases were transferred to clean tubes and evaporated under a stream of dry nitrogen. Each sample was reconstituted with 1 ml of methylene chloride and aliquots were injected into the gas chromatograph equipped with a flame ionization detector (Beckman Instrument, Fullerton, CA, USA). Separation was effected on a 1.83 m x 4 mm i.d. glass column, packed with 3% OV-17 on 80/100 Gas-Chrom Q (Pierce Chemical, Rockford, Illinois, USA). The column temperature was 190° C, the injection temperature was 255° C, and the detector was set at 285° C. The carrier gas was nitrogen with a flow rate of 60 ml/min.

Calibration curves over the range of 1-5 mg/ml diazepam concentrations were prepared by plotting peak height ratio (PHR) of diazepam to internal standard vs diazepam

concentration. The quantity of diazepam present was determined by reference to the calibration curve.

Bioavailability Study

Three mongrel dogs were used to evaluate the IM bioavailability of diazepam from formulations XXV and XXX with reference to the Valium injection. The formulations were administered using a randomized, three-way complete cross-over study design. The diazepam was administered into the biceps femoris muscle at a dose of 2 mg/kg. A catheter fitted with a Heparin Male Slip Adapter was inserted into the foreleg cephalic vein of the dog for blood sampling. Serial 1 ml blood samples were collected at 0, 5, 7, 10, 15, 30, 45, 60, 90 min, and 2, 3, 4, 6, 8, and 12 h in vacutainers following administration of diazepam. No food was given to the animals until the end of study, but water was provided *ad libitum*. The formulations were administered with a washout period of one week between each treatment.

Plasma was separated from the blood by placing Sure Sep-II separators (General Diagnostics, NJ, USA) on the top of the vacutainers and centrifuging at 4000 rpm for 10 min. Clear plasma was poured off, frozen immediately and stored until the analysis of diazepam within a week.

Analytical Method for the Bioavailability Study

The plasma samples were analyzed for diazepam using a "high-performance" liquid chromatography method of Vree et al. (21). Peak height ratios for diazepam to internal

standard (flunitrazepam) were determined manually. The concentrations of diazepam in plasma samples after administration of diazepam were determined from a linear regression of standard curve constructed as PHR vs known concentrations of diazepam in drug free plasma.

Data Analysis

The data were analyzed by noncompartmental methods (22). The elimination rate constant for diazepam was determined from the slope of the terminal monoexponential decline in log plasma concentrations vs time plot by the linear regression method. The peak plasma concentrations (C_{max}) and peak times (t_{max}) were determined by visual observation. Area under the plasma diazepam concentration vs time curve (AUC) was calculated by the trapezoidal rule from time 0 to time t of the last sample and extrapolated to ∞ according to tail correction. Relative bioavailability (F) was determined from the ratios of AUCs of XXV and XXX to Valium.

Statistical Tests

The differences in mean pharmacokinetic parameters for Valium, XXV, and XXX were determined using a two-way ANOVA procedure using SAS package at the significance level of 0.05.

RESULTS

Parenteral diazepam formulations were observed visually for the appearance and the precipitation of the drug. The clear formulations over a period of one week were selected for

further evaluation. All formulations developed a yellow color within one week. Formulations XXV and XXX were selected for further investigation based on the composition of solvent system and visual clarity. The composition of solvent systems for diazepam 10 mg/ml is summarized in Table 1.

The average viscosity of diazepam formulations XXV and XXX was found to be 9.72 and 11.13 Cps., respectively using Stormer viscosimeter at 22° C. The increase in amount of PEG 400 was found to increase viscosity of the diazepam formulations.

Accelerated Stability Studies

The log concentrations of diazepam remained vs time plots over a period of 30 days were linear at temperatures 80, 85, 90, and 95° C. The loss in diazepam content was a first order process and can be described by

$$\log C = \log Co - \frac{k.t}{2.303} \qquad (1)$$

where,

C = concentration at any time t (mg/ml)

Co = initial concentration (mg/ml)

k = first order degradation rate constant (day^{-1})

The degradation rate constant, k was calculated from the slope of linear least square regression analysis of log concentrations vs time data at each temperature.

The shelf-life of the formulations at 25° C was calculated using the Arrhenius equation (23):

$$k = S.\, e^{-\left(\frac{Ha}{RT}\right)} \qquad (2)$$

where,

Ha = energy of activation (calories/mole)

R = gas constant (1.987 calories/degree/mole)

T = absolute temperature (° K)

S = frequency factor (/time)

Arrhenius plots for diazepam in formulations XXV and XXX are shown in Figure 1. Since the plots of log k vs 1/T were linear, the prediction of shelf-life at room temperature is possible by extrapolating the curve to the lower temperature.

The stability half-life ($T_{1/2}$) of diazepam at room temperature was calculated by extrapolation of log k vs 1/T plot and was found to be 870 and 1124 days for formulations XXV and XXX, respectively. The shelf-life ($t_{10}\%$) of these formulations was calculated by (23)

$$t_{10}\% = 0.152.\ T_{1/2} \qquad (3)$$

The shelf-life values of formulations XXV and XXX were calculated to be 132 and 171 days, respectively.

Bioavailability Study:

The concentration of diazepam for formulations XXV and XXX was selected as 10 mg/ml instead of 5 mg/ml of the Valium. The purpose of this change was to minimize the volume of nonaqueous solvent to be injected into the body. The log plasma concentrations vs time plots of diazepam in the dog after IM injection (2 mg/kg) of Valium, XXV, and XXX are shown in Figure 2. Plasma diazepam concentration increased rapidly after IM injection of these formulations. Therefore, the

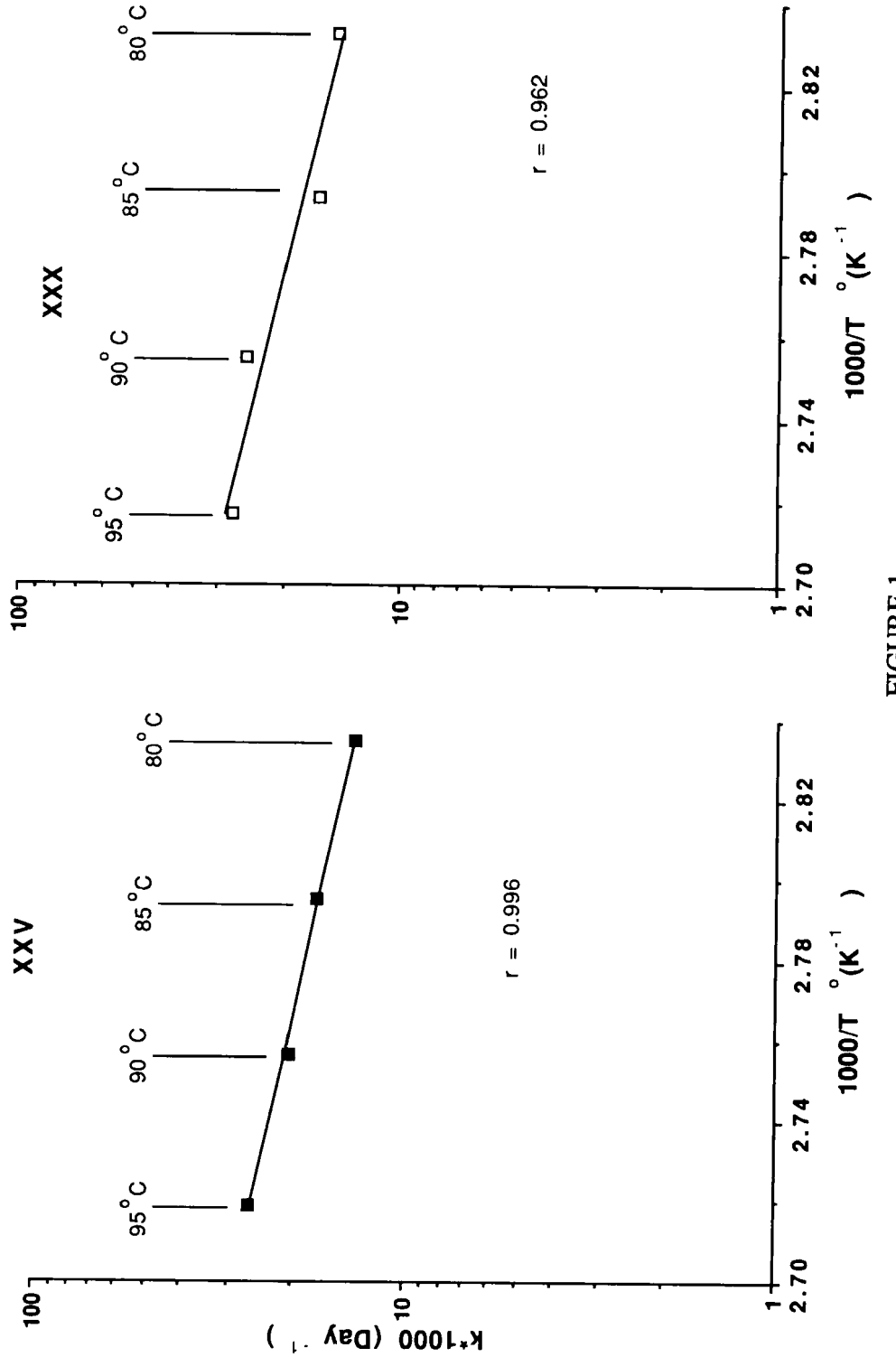

FIGURE 1

Arrhenius Plots for diazepam in formulations XXV (■) and XXX (□)

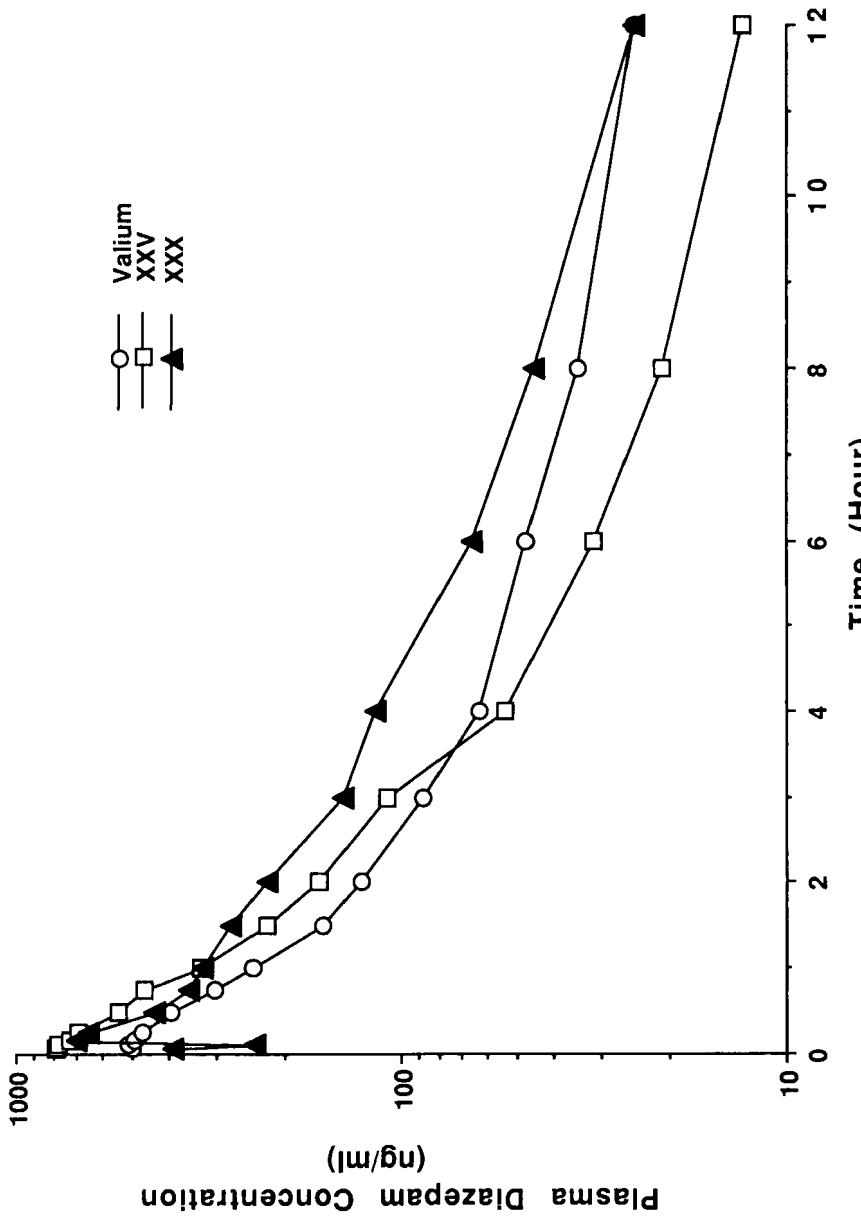

FIGURE 2

Log plasma mean concentrations of diazepam after intramuscular administration (2 mg/kg) of Valium (O), XXV (□), and XXX (▲) to dogs.

TABLE 2

Pharmacokinetic Parameters of Diazepam After Intramuscular Administration of Diazepam (2 mg/kg) to Dogs

Formulation	Dog #	AUC (ng.hr/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	F
	1	1289	340	0.17	8.4	--
Valium	2	1600	754	0.17	8.0	--
	3	997	447	0.25	3.6	--
Mean		1298	514	0.19	6.5	--
SD		301	215	0.05	2.7	--
	1	1170	947	0.08	2.9	0.91
XXV	2	1242	708	0.08	4.6	0.77
	3	1392	881	0.17	4.1	1.40
Mean		1268	845	0.11	4.1	1.03
SD		113	124	0.05	0.6	0.33
	1	1574	807	0.17	4.4	1.22
XXX	2	2138	1051	0.17	3.5	1.34
	3	1068	297	0.25	2.9	1.07
Mean		1594	718	0.19	3.6	1.21
SD		535	385	0.05	0.8	0.14

absorption rate constant could not be determined. The pharmacokinetic parameters after IM administration of diazepam in Valium, XXV, and XXX are shown in Table 2.

The peak plasma concentrations were achieved within 15 min with all formulations. The peak plasma concentrations

for XXV and XXX were 39.2% and 28.2% higher than that for Valium, respectively but were not significantly different ($p>0.05$).

The AUC for diazepam in XXX was 18.6% and 20.5% higher than that for Valium and XXV, respectively. However, the differences in AUCs for the above three formulations were not significant ($p>0.05$). The half-life ($t_{1/2}$) of diazepam in the dog ranged from 2.9 to 8.4 h. Relative bioavailability of formulations XXV and XXX was 1.03 and 1.21, respectively compared to the Valium injection.

DISCUSSION

Diazepam is practically insoluble in water. Nonaqueous solvents are used to make parenteral solutions of diazepam. The commercially available injectable diazepam preparation, Valium, is formulated with a combination of PG and ethanol to make a 5 mg/ml solution of diazepam. Intravenous injection of this solution causes a high incidence of venous adverse reactions including thrombophlebitis and phlebothrombosis (4-6). Both diazepam and nonaqueous solvent PG have been implicated in causing adverse venous reactions (4, 7-9). Polyethylene glycol 400 has been suggested as an alternative solvent for drugs which do not have a high degree of water solubility (24). This solvent seems to have a lower incidence of adverse effects than PG (11) although comparative studies have not been carried out. Kortilla et al. (25) have reported a significant reduction in pain after IM injection of diazepam in

humans when PG was replaced with PEG 300 without influencing the bioavailability. In the present investigation we prepared parenteral diazepam solutions by either reducing or replacing PG of the commercial preparation, Valium with PEG 400.

The formulation XXV contained the minimum amount of nonaqueous solvent system (68.4%) necessary to dissolve 10 mg of diazepam per ml. By replacing all of PG in XXV with PEG 400 in formulation XXX also resulted in a clear solution of diazepam. The formulations used in this study were prepared with a concentration of 10 mg/ml as opposed to 5 mg/ml for the Valium. The higher concentration of diazepam (10 mg/ml) in formulations XXV and XXX was intended to decrease the volume of nonaqueous solvent system to be injected into the body e.g., administration of a 5 mg (1 ml) of Valium will introduce a 0.5 ml (50%) of nonaqueous solvent into the body while administration of the same amount of diazepam from either formulations XXV or XXX will decrease the volume of nonaqueous solvent to 0.34 ml (34%). The decrease in volume of nonaqueous solvents may reduce pain at the site of injection and also prevent the muscle injury if the solvent system is responsible for these adverse reactions (13, 26).

Since diazepam is photosensitive (3, 27), all formulations developed a yellow color within fifteen days of storage at room temperature and exposed to light. However, there was no measurable loss in diazepam content due to this change. It has

been recommended that diazepam injection should be stored in a container protected from light (3).

The viscosity of formulations XXV and XXX was found to be 9.32 and 11.13 Cps., respectively using Stormer viscosimeter at 22° C. The commercial Valium has a viscosity of 5.0 Cps. (28). The higher viscosity of the formulations used in this investigation was mainly due to the PEG 400. The viscosity of PEG 400 and PG at 22° C was found to be 82.22 and 32.85 Cps., respectively.

The expected shelf-life of formulations XXV and XXX using equation 3 resulted in values of 132.2 and 170.9 days, respectively. These estimates were shorter than the results obtained from the stability studies at room temperature in which less than 10% loss in diazepam content was detected in formulations XXV and XXX at 18 and 12 months, respectively after preparation (data not shown). The commercially available Valium injection has a shelf-life of 2 years (28). Valium injection has a pH between 6.7-7.3. The formulations prepared in this investigation had a final pH of 4.5. The differences in pH between the Valium and the formulations used in this study may not explain the differences in the shelf-life since diazepam has been reported to be stable between the pH of 4-8 (28). It is recommended that for the precise estimate of shelf-life of a preparation, stability studies at room temperature should be carried out at least over a period of 2 years (23).

In the present study, the absorption of diazepam after IM injection into the dog muscle was rapid and the absorption phase was complete after 15 min. The volume of injection for Valium was twice as much as that for formulations XXV and XXX. However, the AUC and the C_{max} for formulations XXV and XXX were not significantly different from the corresponding parameters for the Valium ($p>0.05$) suggesting the volume of injection did not have effect on these parameters in the dog. Similarly, the higher viscosity of XXV and XXX compared to the Valium did not alter the rate and extent of diazepam absorption after its IM injection. Pharmacokinetic parameters for diazepam estimated in the present investigation are in good agreement with previously reported literature values (29, 30). In this study, considerable inter- and intra-animal variation in the half-life was observed.

CONCLUSION

In this pilot study formulations XXV and XXX were found to be bioequivalent to Valium after IM administration into the dog. Reduction in the volume of injection and the replacement of PG with PEG 400 without influencing bioavailability with formulation XXX could be advantageous. Pain at the site of injection and fatigue after IM injection of Valium are problems which may be minimized with the new formulation. Further investigations of this new preparation are necessary to prove these claims.

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