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Full Length Research Paper

In vitro and *in vivo* evaluation of two sustained release formulations of diltiazem HCl

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In the present study, two sustained release solid and semi-solid matrices were developed using Hydroxypropyl methyl cellulose (HPMC) and Gelucire derivative, Gelucire 50/30. The purpose of the study was *in vitro* and *in vivo* correlation of these two sustained release formulations with SR capsules available in market and to know that for how long the preparation containing HPMC and Gelucire work in the body as compared to the product circulating in the market, so for this two formulations were developed such as solid matrices in tablets form and semi-solid capsules. For the preparation of solid matrices, direct compression method and for semi-solid, filling capsule technology were used. *In vitro* and *in vivo* study was perfomed and different parameters were studied such as Cmax, Tmax and AUC for all the three formulations. For determination of Cmax, Tmax ans AUC statistical models were used. *In vitro* study showed that more than 80% drug was released upto 12 h from all the three formulations and no significance difference was observed in release pattern while *in vivo* study showed prolonged release of the two test formulations after applying statistical models. The drug release from both test formulations was slow thereby providing a prolonged and controlled *in vivo* delivery of the drug. This proved the superiority of our test capsules and tablets over the reference capsules.

Key words: *In vitro* and *in vivo* correlation, deltiazem, kinetic models, statistical analysis.

INTRODUCTION

As for the availability of a drug over extended period of time after administration different extended release dosage forms are developed and formulated. For the description of extended release dosage forms different expressions have been used such as controlled-release, prolong–action, repeat action and sustained-release. The purpose of developing such systems is to provide constant or nearly constant drug levels in plasma with reduced fluctuations because of slow release over an extended period of time. The sustained release formulations are developed of those drugs which have low elimination half life and low therapeutic indices (George et al., 1978). Sustained release formulations are achieved by using different approaches such as coating technology, slow eroding devices, osmotically controlled devices and matrix systems of swellable or nonswellabe polymers. In the present work, matrix system has been used because matrix tablets are easily formulated and not expensive and their release pattern is also appreciable (Mishra et al., 2003). For the development of sustained release formulation different polymers are used and those polymers are more suitable for the formulation and designing of sustained release matrices which have the retarding capability (Nellore et al., 1998). So for the development of solid and semi-solid matrices, Hydroxypropyl methyl cellulose (HPMC) and Gelucire 50/13 were used, as hydroxypropyl methyl cellulose (HPMC) is a hydrophilic polymer which is used for the formulation of sustained release matrices (Behl and Dhake, 2005). It is mixed alkyl hydroxyl cellulose and with the increase of hydroxypropyl content the hydration rate is also increased and its solubility is pH dependent and it is widely acceptable excipient (Alerman, 1984). Gelucires are also used for the controlled or sustained release formulation and these are derived from a mixture of mono-di-triglycerides with poly ethylene glycol esters of fatty acids (Ainaoui and Vergnaud, 1998; Sheu and Hsia, 2001). Gelucire 50/13 is the member of

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Table 1. Composition of solid and semisolid test matrices.

Formulation	Diltiazem HCL (mg)	HPMC (mg)	Gelucire 50/13 (mg)
Solid matrix	90	210	-
Semisolid matrix	90	-	210

Gelucires family which is mostly used for the preparation of sustained release formulation (Dennis et al., 1990).

Deltiazem HCL is a potent drug related to the calcium channel blockers used for the treatment of hypertension, arrhythmia and for the management of angina pectoris and its half life is 3.5 h and its dose is 30 mg t.i.d, so to improve patient compliance and reduce side effects and dosage frequency it is a suitable entity for the formulation of sustained release dosage form (Chaffman and Brogden, 1985).

MATERIALS AND METHODS

Materials

The following materials were used; Diltiazem HCl (Novartis, Pakistan), Verapamil HCl, Herbesser-SR90mg (Highnoon Laboratories, Pakistan) Batch No: 0806, (Mfg.Date,01/2008 and Exp.Date 01/2010), Cyclohexane (Merck, Germany), Acetonitrile (Merck,Germany) ,Diethyl ether(Norway), HPMC K15M (Colorcon, Karachi, Pakistan), Disodium Hydrogen Phosphate, HPLC Grade (Merck, Germany), PotassiumDihydrogen Phosphate, HPLC Grade (Merck, Germany), Sulphuric Acid, HPLC Grade (Merck, Germany),Ortho Phosphoric Acid, HPLC Grade (Merck, Germany),Triethylamine(Fluka,Switzerland)Methanol, HPLC Grade (Merck, Germany)

Instruments

The following instruments were used during experimental work. Hot plate magnetic stirrer (Velp Scienifca, Germany), UV-Spectrophotometer (Shimadzu 1601, Japan), pH meter (Inolab, Germany), Digital,weighing balance (Precisa, Switzerland), Dissolution apparatus USP (Pharma Test, Germany), Oven (Mammert, Germany),Friabiliator (Emmay, Lahore), Digital Hardness tester (Curio, Germany),Vaccum Filter Assembly (Sartorius Goettingen, Germany), Cellulose Acetate Filter 0.45 µm (Sartorius Goettingen, Germany), HPLC (Perkin Elmer Series 200, USA), Water Distillation, Appratus (IM-100, IRMECO GmbH, Germany), Whatman Filter Paper (Whatman, Germany), Vacuum Pump (ILMVAC-Germany), Vortex Mixer (Seouline BioScirnce-Korea), Centrifuge Machine (Helttich, Germany), Centrifuge Tubes (pyrex france), Disposable Syringes (BD pakistan), Reacti vials (Greiner lavortechnik-Germany), Sonicator (ElmaD78224, Germany).

Methods

Preparation of matrix tablets

Direct compression method was used for the preparation n of solid matrix tablets by using Single punch tablet machine (Emmay, Lahore, Pakistan) and all ingredients were First mixed well in polythene bags and tablets of 300 mg were prepared as shown in Table 1 and then different physical and dimensional tests applied and all were in acceptable range.

Preparation of semi-solid matrices

The weighed amount of Gelucire 50/13 as shown in Table 1 was heated in a beaker at 70°C for 1 h after this the weighed amount of diltiazem HCL was added to the molten Gelucire using hot plate magnetic stirrer (ARE, Europe) by fixing stirring speed at 500 rpm and temperature 70°C for one hour. Once a homogeneous mixture was obtained the resultant viscous material was filled into hard gelatin capsule using pasture pipette. Capsules were stored in a tightly closed jar until used for dissolution.

In vitro dissolution studies of solid and semi-solid matrix system

In Vitro study was performed by USP methods 1(basket method) and method 2 (paddle method) using Pharma test apparatus (Germany) and the study was performed in 900 ml different dissolution media that is, water, pH 1.0 (0.1 M HCL), pH 4.0 and pH 7.0 (phosphate buffer) and temperature was maintained 37.0±0.5°C and the stirring speed was set at 50 rpm. Sample of about 5 ml each was collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0,10 and 12 hours in a glass tubes after filtering through 10µm Sinter filters and same amount were replaced by fresh medium. An aliquot of about 0.5 ml from each sample was drawn and diluted to 25 ml with distilled water and analyzed at 237nm using UV/Visible double beam spectrophotometer (Shimadzu 1601, Japan). Percentage drug releases at different sampling intervals were calculated by using calibration curve .All the tests were run in triplicate and average was taken.

Physical and dimensional tests

In order to determine the uniformity in weight of capsules and tablets, twenty tablets were weighed using class a weight balance (Precisa, Switzerland) and their percentage variation was determined. The weight variation of all capsules and tablets was well within the acceptable limits of BP (2002) indicating that the filing of the shells in case of capsules and die cavity in case of tablets was uniform. The deviation in both the test formulations was not greater than 3%. Hardness of tablets was also determined using automatic hardness tester (Curio, Pakistan). Ten tablets were used and the average hardness value was determined.

The tablets were also subjected to friability test employing friabilator (Emmy, Pakistan). Twenty tablets were placed in tumbling chamber and rotated precisely for 4 min at a speed of 25 rpm. The weight of twenty tablets prior to their placement in the chamber and at the end of the test was recorded. The percentage weight loss was then calculated. Triplicate measurements were conducted for tablet. The acceptable limit of weight loss was not more than 0.8%.

Assessment of in vitro drug release data using various kinetics models

Drug release kinetics is assumed to reflect different release

Table 2. Dosing schedule of two test and reference formulations in three way cross over study.

	Groups		
	G1	**G2**	**G3**
Week A	Test tablet	Test capsule	Reference capsule
Week B	Test capsule	Reference capsule	Test tablet
Week C	Reference capsule	Test Tablet	Test capsule

mechanisms of controlled release matrix systems. Therefore, different kinetics models were applied to analyze the *in vitro* release mechanism that is, zero-order release Equation (1), Higuchi equation (2) and first-order equation (3).

$$Q = k_1 t \quad (1)$$

$$Q = k_2 (t)^{0.5} \quad (2)$$

$$Q = 100(e^{k3t}) \quad (3)$$

Where Q is the percentage release at time t, k_1, k_2 and k_3 are the release rate constants for zero-order, Higuchi and first order model, respectively(Merchant al el, 2006) derived an equation to determine the drug release mechanism as shown in equation 4.

$$M_t/M_\alpha = k_4 t^n \quad (4)$$

Where Mt/M_α is the fraction of drug released at time t, k_4 is the kinetic constant and n is the so called diffusion exponent, indicative of the mechanism of the drug release.

The equation generally holds for $M_t/M_\alpha > 70\%$ of drug release. N = 0.45 or 0.45 < n < 0.89 or n > 0.89, indicates Fickian diffusion or anomalous transport or Case ''II'' transport kinetics respectively (Peppas, 1985). Regression analysis was performed to obtain the release rate constant and the values of coefficient of regression *(R^2)* were compared.

In addition, the similarity factor f_2 (Shah et al., 1999) and is used to compare the difference of dissolution profile between the commercial product and experimental formulation

$$F_2 = 50\text{Log}\{[1+ 1/n \sum (R_t-T_t)^2]^{-0.5} \times 100\}$$

Where n is the number of dissolution sample times and R_t and T_t are the individual percentages dissolved at each time point, t for the reference and test dissolution profiles, respectively. f_2 value greater than 50 (50-100) represent equivalence of the two dissolution curves.

In vivo study protocol

For *in vivo* study total twelve volunteers were selected and the protocol used was a conventional, three-way, split group, crossover study with 4 subjects in each of the three treatment groups. The preparations were administered according to the schedule shown in Table 2.

In the first trial period, each volunteer in first group was given one test tablet 90 mg, the second group was given one test capsule 90 mg and third group was given one reference capsule of Herbesser 90 mg as shown in Table 2. After a washout period of one week, each group of volunteers then received the alternate product. The three preparations were administered in the morning at 9.00 a.m. to fasting subjects with 240 ml of water in an attempt to reduce variability. Food and drinks were withheld for at least 2 h after dosing. Blood samples of 5 ml volume were collected in centrifuge tubes (containing sodium heparin as anticoagulant) at 0 (before dosing), 0.5, 1.5, 2, 3, 4, 6,8,10, and 12 h after dosing via an in-dwelling cannula placed in the forearm. Blood samples were also collected after 24 h via direct puncture with 5 ml syringe. The blood samples were centrifuged for 15 min at 3500 rpm and the plasma was transferred to new glass tubes and kept frozen at -20°C until analysis.

Analysis of plasma by using HPLC

The plasma samples were analyzed using a reversed-phase high-performance liquid chromatographic (HPLC) method. The HPLC system comprised of an HPLC (Perkin Elmer Series 200, USA) with TCNav software, consisted of binary pump solvent delivery system, an ultraviolet-visible (UV-Vis) variable wavelength detector, integrator NCI 900 was used. Samples (20 µl) were introduced into a rheodyne fixed-loop injector with a 50 µl glass syringe. Chromatographic separation was performed on a partition 5 µm pore size, 4.0 × 250 mm ODS Hypersil C18 stainless steel analytical column (Thermo Electron Corporation, UK) fitted with a refillable guard column. All solvents were degassed by Sonicator (Elma D78224, Germany) and the pH of mobile phase was adjusted by pH meter (Inolab Series, Germany) prior to use.

The mobile phase consisted of 0.05 M sodium hydrogen phosphate dihydrate: acetonitrile (63:37 v/v). Triethylamine (0.1%) was added before the pH was adjusted to 5 with 85% orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter (Sartorius U.S.A) and was then degassed by ultrasonication. Analysis was run at a flow rate of 1.0 ml/min and the detection wavelength was 237 nm. Quantification was done by peak height.

Extraction procedure

750 µl of the plasma sample was measured into a glass tube with Teflon lined screw cap, followed by adding 50 µl of verapamil HCl as internal standard (5 µg/ml in methanol) and 3 ml of 1:1 n-hexane-diethyl ether mixture was used as extracting solvent. The mixture was then vortexed for 1 min by using a vortex mixer (Seouline BioScience-Korea) and centrifuged at 3500 rpm for 15 min by centrifuge machine (Kubota Japan). After centrifugation the upper organic layer was transferred into React vial (Greiner lavortechnik-Germany) and then solvent was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted with 40 µl of mobile phase and 20 µl injected into column.

Standard solutions

Stock solutions were prepared by dissolving 50 mg of diltiazem HCl and internal standard (verapamil HCl) separately in 50 ml methanol to give a final concentration of 1 mg/ml. Then 1 ml from the above diltiazem stock solution (1 mg/ml) was taken in 50 ml volumetric

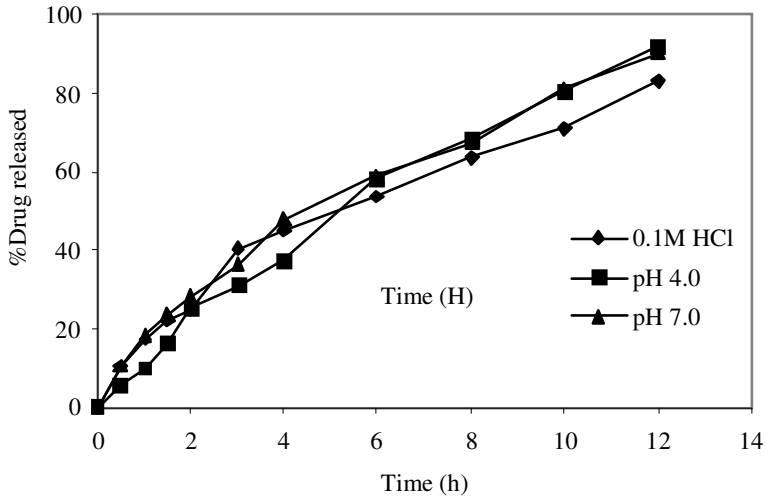

Figure 1. Release pattern of solid matrix tablets in different solvents.

flask to make a final concentration of 20 µg/ml. 0.25 ml of internal standard solution (1 mg/ml) was measured in 50 ml volumetric flask to make the required concentration (5 µg/ml).

Standard curve was constructed to encompass the entire range of plasma diltiazem HCl concentration found in healthy volunteers. Blank plasma was spiked with known amount of diltiazem HCl to give concentrations of 6.25, 12.5, 25, 50, 100, and 200 ng/ml. The standard plasma samples were stored at -20°C in the glass bottles. Recovery, precision and accuracy studies were carried out using these plasma standards. In addition, detector linearity was determined with diltiazem HCl standard solutions prepared in acetonitrile over a concentration range of 6.25 – 200 ng/ml.

Data analysis

The most common pharmacokinetic parameters such as total area under the plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were estimated from the plasma concentration-time profiles of the three preparations for each volunteer. The C_{max} and T_{max} values were obtained directly from the plasma-concentration data (Weiner, 1981). The $AUC_{0-\infty}$ was calculated by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling time to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula and the latter by dividing the last measurable plasma drug concentration with the apparent elimination rate constant (k_e). In all cases, the $AUC_{t-\infty}$ was found to be less than 10% of the AUC_{0-t}. The k_e was estimated from the terminal slope of the individual plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression (Gibaldi and Perrier, 1982). On the other hand, the elimination half-life ($t_{1/2}$) was calculated from the quotient $\ln 2/k_e$, while the apparent volume of distribution (Vd) was calculated as Dose/($AUC_{0-t} \times k_e$) .The *in vivo* absorption profiles of the formulations were also calculated from the individuals plasma concentration versus time data using Wagner-Nelson (1964) method. The equation used for the determination of individual absorption profile is as follows,

$$\% \text{ absorbed at time } t = \frac{Ct + k_e \, AUC_{0-t}}{k_e \, AUC_{0-\infty}} \qquad (2.6)$$

Where, Ct is the plasma concentration at time t, k_e the elimination rate constant, AUC_{0-t} the area under the plasma concentration time curve from time zero to time t and $AUC_{0-\infty}$ the total area under the plasma concentration versus time curve. In addition, correlation between the *in vitro* dissolution and *in vivo* absorption times for 20, 40, 60 and 80% of drug released/absorbed was determined for the three preparations using simple regression and correlation analysis.

Statistical analysis

The calculated values of pharmacokinetic parameters $AUC_{0-\infty}$, C_{max}, t½, k_e and Vd obtained with the three preparations were analyzed statistically using an analysis of variance (ANOVA) procedure which distinguished effects due to subjects, periods, and treatment (Wagner, 1975).The $AUC_{0-\infty}$ and C_{max} values were logarithmically (log) transformed prior to the analysis. On the other hand, the T_{max} values of the three preparations were analyzed using the paired sample t-test. A statistically significant difference was considered when $P < 0.05$. In addition, the 90% confidence interval of the ratio of Logarithmic transformed AUC_{0-t} as well as C_{max} of the test formulation over those of the reference capsules was also determined.

RESULTS AND DISCUSSION

In vitro evaluation

The *in vitro* dissolution studies of two sustained release test formulations of diltiazem 90 mg (matrix tablets and semisolid capsules) and reference products (Herbesser 90 mg-SR, pellets filled capsules) were performed by using various dissolution media such as 0.1 M HC, pH 4.0 and 7.0 phosphate buffer solutions as shown in Figures 1, 2 and 3 and the same study was performed in water and the released pattern was observed as shown in Figure 4. The slight differences in the release profile of the three formulations in various dissolution media are visible. More than 80% drug was released upto 12 h from all the three formulations and the release of drug from tablets and capsules was similar as that of reference- SR

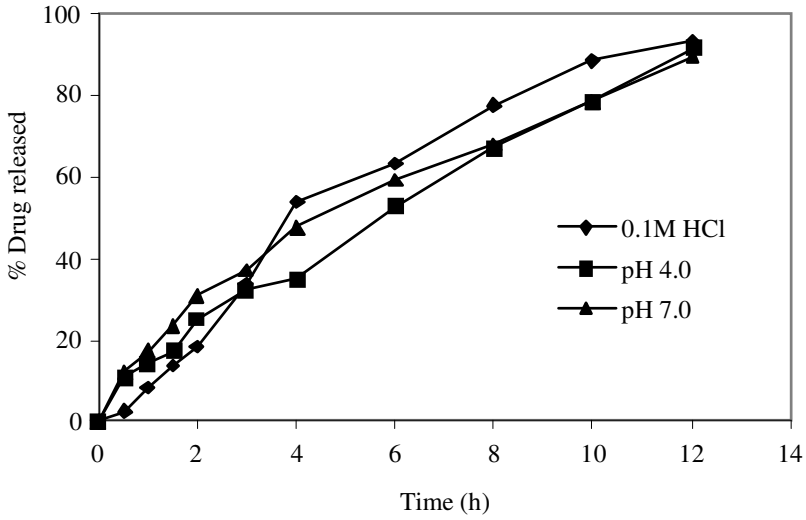

Figure 2. Release pattern of semisolid formulation in different solvents.

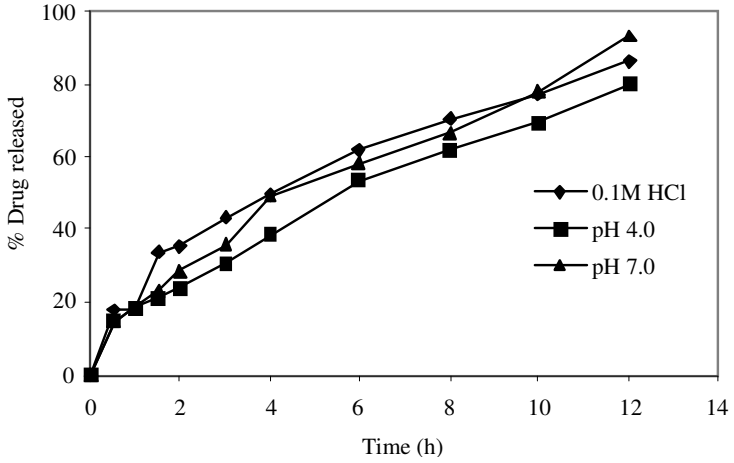

Figure 3. Release pattern of reference in different solvents.

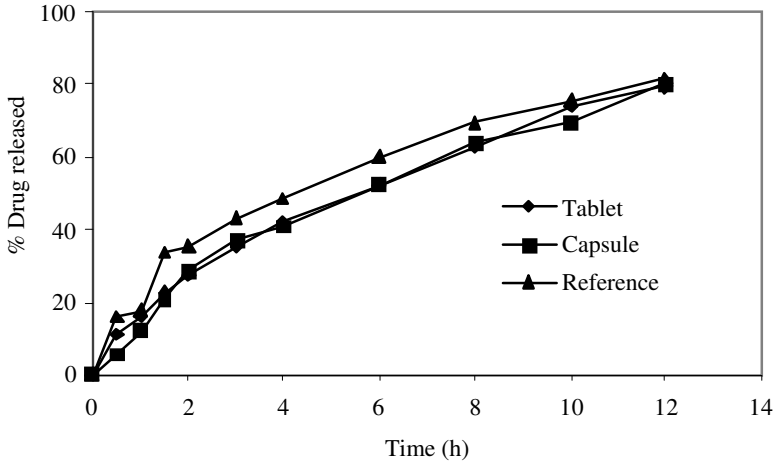

Figure 4. Release pattern of all three formulation in water.

Table 3. Kinetic release mechanism of all three formulation.

Models	Reference capsules		Test capsule		Test tablet	
	R^2	$K(\%hr)^{-1}$	R^2	$K(\%hr)^{-1}$	R^2	$K(\%hr)^{-1}$
Zero Order	0.929	3.28	0.947	4.324	0.9798	6.934
First Order	0.55	0.193	0.737	0.292	0.6	0.239
Hixson Crowell	0.953	-0.062	0.962	-0.081	0.965	0.183
Heguchi	0.993	12.82	0.944	16.301	0.969	26.054
Korsmeyer-peppas	0.569	0.767	0.905	1.256	0.622	0.945

capsules. Both the test formulations with two different matrix systems follow the same release characteristics. HPMC based test matrix formed gel layer around the surface of the tablets during dissolution because HPMC form gel, possessing high strength (Nicole and Owen, 2004) and drug diffuses in dissolution media by diffusion process. But when water was used as dissolution media for semi-solid test matrix system, there was no gelling of the polymer due to inability of the polymer to accommodate water uptake. Instead the semi-solid matrix system seemed to be disintegrated and dissolved completely after 12 h. It is because of early brusting of capsule shell and more surface area of capsules expose to the madia. Both the test formulations, formulated by using two different matrix systems followed the same release profile.

The release mechanism was evaluated using different kinetic models. The drug release rate constants (K) and regression coefficient (R^2) obtained from Zero, First order, Higuchi, Hixson Crowell and Korsmeyer-Peppas models as shown in Table 3. It is apparent that the dissolution data of the three formulations could be better fitted in Higuchi model as the R^2 values of the three formulations were found to be greater as compared other kinetic models. Moreover, when in vitro release profiles of three formulations were fitted in f_2 equation for similarity, the values obtained were above 50 indicating that the three formulations are comparable with each other.

In vivo study

Twelve (12) healthy non-smoking adult male subjects between 20 and 28 years old (Mean = 22.8 years, SD = ± 2.8 years), with heights from 162.5 to 174.4 cm (Mean = 162.5 cm, SD = ± 4.0 cm), and weighing from 53 to 81 kg (Mean =57.9 Kg, SD = ± 3.1 Kg), were participated in the study. Written informed consent was obtained from the volunteers after explaining the nature and purpose of the study. All were judged to be healthy on the basis of their blood and urine reports and were not receiving any medication during the study period.

Analysis of plasma

Several HPLC methods have been reported for the analysis of Diltiazem HCl and some of the methods involve liquid-liquid extractions with slight differences in the extracting solvent mixtures, the mobile phase, the size of the column and the total time for analysis. A single stage extraction method with minimum extraction time and analysis time was developed from the reported methods (Nisar et al., 2006; Al-Saidan, 2005). The reported method was slightly modified by changing the composition of mobile phase consisting of 0.05 M sodium hydrogen phosphate dihydrate: Acetonitrile: triethylamine (63, 37, 0.1%) was utilized in the present study for good separation of compounds with sharp peaks within 12 min.

The retention time of Diltiazem HCl and internal standard (Verapamil HCl) is 7.28 and 11.77 respectively. The blank sample was clean and no interfering peak was observed at the retention times of Diltiazem HCl, and there is no interference between the peaks of Diltiazem HCl and internal standard. The extraction recovery of Diltiazem HCl was determined by comparing the peak height obtained by direct injection of standard aqueous solutions to those obtained after the plasma extraction procedure. The recovery values for Diltiazem HCl were more than 90% at 6.25 and 200 ng/ml. The sensitivity of the assay method was approximately 3 ng/ml. The mean plasma standard curve was found to be linear over the concentration range used (Figure 17) with correlation coefficient of 0.9964.

In vivo performance of two test and reference formulations

Individual volunteer plasma Diltiazem HCl concentration versus time profiles of the test capsules, tablets and reference capsules Herbesser-SR (pellets filled) obtained from plasma concentration data of three formulations are shown Tables 4, 5 and 6 and their plasma release profiles are shown in Figures 5 - 16. Whilst the mean plasma Diltiazem HCl concentration versus time profiles of two test formulations and reference product is shown in Figure 17. Slight differences in the individual subject as well as in their mean plasma profiles are apparent and the three formulations are seemed to be comparable. The three *in vivo* profiles are reflective of a slow and sustained rate of drug absorption. Plasma concentrations of Diltiazem HCl were detectable during 24 h from the

Table. 4. Plasma concentrations of individual subjects after administrating diltiazem reference capsules (Herbesser-SR 90 mg).

	Plasma concentrations (ng/ml)													
Time(h)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**mean**	**S.D**
0.50	29.79	9.85	9.41	10.68	10.11	8.38	6.61	10.49	11.67	9.99	9.21	9.81	11.33	5.69
1.00	70.84	29.91	40.74	35.30	30.70	45.51	47.10	35.20	42.44	33.43	35.22	47.08	41.12	10.70
1.50	84.04	67.27	69.67	71.73	84.45	74.92	69.18	77.97	74.97	59.38	63.94	70.51	72.33	7.15
2.00	107.18	97.14	82.01	96.74	107.39	97.60	88.74	110.79	111.56	78.36	83.21	101.46	96.85	11.05
3.00	79.24	106.84	101.73	131.55	100.58	110.13	101.50	87.75	133.35	106.92	124.94	114.73	108.27	15.57
4.00	65.53	104.05	87.20	103.01	97.27	100.48	95.66	70.14	99.94	92.32	97.35	93.16	92.18	11.81
6.00	48.31	90.95	65.73	72.91	87.07	87.14	71.95	54.40	83.06	79.58	73.21	74.83	74.10	12.46
8.00	41.9	71.9	47.8	56.0	64.4	45.6	51.9	52.7	66.3	64.9	61.1	56.1	56.7	8.8
10.00	21.8	54.0	36.3	25.6	33.4	21.9	28.6	27.4	46.4	40.9	33.1	38.2	34.0	9.4
12.00	16.0	28.2	21.6	13.1	21.7	10.4	15.0	18.3	19.9	26.9	21.9	23.9	19.7	5.2
24.00	9.0	8.5	7.3	10.2	8.2	5.8	5.1	7.7	6.6	11.8	7.7	7.6	8.0	1.8

Table 5. Plasma concentrations of individual subjects after administrating diltiazem test tablet (90 mg).

	Plasma concentrations (ng/ml)													
Time(h)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**Mean**	**S.D**
0.50	21.4	9.8	13.8	10.1	8.6	8.0	7.6	11.2	10.3	10.2	13.7	13.0	11.5	3.6
1.00	41.0	36.7	44.1	35.7	37.2	38.2	40.4	29.7	41.1	33.6	40.1	42.6	38.4	3.9
1.50	57.9	63.2	59.6	63.9	66.3	63.1	70.3	64.1	74.2	56.6	63.1	71.1	64.4	5.1
2.00	75.0	89.6	108.5	81.5	84.2	104.9	83.6	78.2	96.7	72.7	91.6	89.9	88.0	10.7
3.00	104.6	109.0	98.0	109.7	110.3	114.7	118.3	103.2	118.7	104.6	115.4	108.9	109.6	6.1
4.00	77.0	104.8	85.4	87.5	89.1	107.7	100.8	86.6	101.7	92.8	93.1	117.8	95.4	10.9
6.00	56.1	93.9	63.0	68.3	70.5	78.7	74.2	69.8	74.6	73.1	67.3	84.5	72.8	9.4
8.00	46.2	70.2	50.7	55.1	50.0	41.4	49.4	52.4	65.1	62.9	56.8	63.3	55.3	8.2
10.00	22.9	56.1	29.2	22.2	28.4	26.4	26.0	28.9	32.1	39.4	29.9	36.6	31.5	8.8
12.00	15.0	26.4	18.6	18.9	14.7	10.8	13.1	18.4	20.3	20.5	20.8	28.1	18.8	4.8
24.00	9.8	6.5	8.2	8.3	7.9	6.6	6.9	7.0	7.0	11.2	6.4	7.3	7.8	1.4

three preparations. Although no lag time was observed in the plasma concentration of the formulations. There was rapid increase in the plasma concentration and reaching maximum at approximately 3 h after dosing, being typical that obtained with sustained release preparations.

The mean *in vivo* Diltiazem HCl absorption profiles versus time of three formulations calculated by using Wagner-Nelson (1964) method are depicted in Figure 18. All the three profiles were comparable and no significant difference in their absorption was found.

Individual numerical and pharmacokinetic values of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max} Ke, $t_{1/2}$, Vd and MRT obtained with pellets filled Herbesser-

Table 6. Plasma concentrations of individual subjects after administrating diltiazem test capsule (90 mg).

Plasma concentrations (ng/ml)														
Time(h)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**Mean**	**S.D**
0.50	20.7	10.6	10.2	10.9	11.6	7.0	5.1	12.8	10.2	14.6	12.1	8.1	11.1	20.7
1.00	52.8	34.8	31.3	43.8	33.8	33.5	45.2	34.9	40.5	39.1	40.0	43.5	39.4	52.8
1.50	76.3	60.4	50.2	67.7	53.9	60.8	69.5	63.1	78.7	64.7	66.4	79.7	66.0	76.3
2.00	103.0	99.5	83.5	85.6	77.9	102.7	97.2	78.2	101.6	78.5	84.7	94.6	90.6	103.0
3.00	91.0	113.6	101.9	114.6	98.2	106.6	121.7	107.7	117.3	109.7	122.3	127.9	111.0	91.0
4.00	68.9	86.5	89.9	102.7	86.3	94.4	97.9	87.4	105.1	84.0	99.7	106.9	92.5	68.9
6.00	64.6	71.6	74.0	78.5	77.9	59.9	77.5	63.2	81.9	67.7	77.2	77.5	72.6	64.6
8.00	38.3	66.8	48.2	43.3	62.2	30.9	54.3	46.4	65.0	51.9	60.4	66.4	52.8	38.3
10.00	18.9	38.7	43.3	28.2	39.3	18.3	32.6	31.4	46.2	34.8	30.3	44.3	33.9	18.9
12.00	15.0	18.9	26.5	13.9	22.0	9.1	15.5	12.9	22.7	26.9	21.6	24.5	19.1	15.0
24.00	6.0	8.1	8.3	8.0	8.8	3.8	7.9	7.5	7.7	10.3	8.1	8.7	7.7	6.0

SR capsules, tested tablets and capsules are presented in Tables 4, 5 and 6. The values obtained from two test formulations and reference was closely similar and not significantly different statistically.

To facilitate bioequivalence comparisons, pharmacokinetic parameters for each volunteer were displayed in parallel for the three formulations. In particular, for AUC_{0-t} the difference between test and reference (T-R), ratio (T/R) and log of ratio (log T/R or Ln T/R) among the tested and reference values were tabulated side by side for all the subjects and shown in Tables 7 - 11.

The parameters $AUC_{0-\infty}$ and T_{max} are related to the respective rate and extent of drug absorption, while C_{max} is related to both processes (Grahnen, 1984). The mean T_{max} values for reference (Herbesser-SR is 2.75 ± 0.45 h) were similar compared to tested Capsule (2.92 ± 0.29 h) and Tablet (3 ± 0.43 h) indicating similar absorption rate. Statistical analysis showed no significant difference between the T_{max} values of Herbesser-SR capsules and test capsule and tablet ($p > 0.05$). T_{max} in the present situation may not be reliable estimate of rate of Diltiazem HCl absorption due to slight fluctuations of peaks in the plasma concentration versus time profile. In the present study, C_{max} for Herbesser-SR capsules was found to be 113.07 ± 10.95 ng/ml compared to 111.43 ± 9.64 ng/ml of capsules and 111.24 ± 5.61 of tablets. C_{max} of the reference was slightly higher than capsule and tablet. Three-way cross-over design was applied on results using SPSS 12.0 software. No significant difference ($P = 0.790$) was found between the values of C_{max} of the three formulations as shown in Table 9. AUC_{0-t} values of Herbesser-SR capsules, test capsules and tablets were 912.20 ± 90.77, 880.97 ± 99.34 and 873.17 ± 91.31 ng.h/ml, respectively. Statistically these values were not significantly different.

In the present study the values of $AUC_{0-\infty}$ for Herbesser-SR capsules, test capsules and tablets were 866.72 ± 118.04, 944.98 ± 110.80 and 933.93 ± 93.90 ng.h/ml, respectively. Relatively wide inter-subject variation was observed in the values of the parameters $AUC_{0-\infty}$ and C_{max} which could be attributed to differences in body weight and drug disposition among the subjects. However, no statistically difference was observed between the log transformed AUC_{0-t} ($P = 0.946$) values, the log transformed $AUC_{0-\square}$ ($P = 0.219$) values as well as log transformed C_{max} values ($P = 0.820$) of the three preparations.

In addition, the 90% confidence interval (CI) for the ratio of the log transformed C_{max} values of test tablet and test capsule over those of Herbesser-SR capsules were estimated to be between 0.94 - 1.03 and 0.94 - 1.04, respectively as shown in Table 16. CI for the ratio of the log transformed AUC_{0-t} values of test tablet and capsule with respect to Herbesser-SR capsule was found to be between 0.96 - 1.06 and 0.91 - 1.08, respectively; while the CI for the ratio of the log transformed $AUC_{0-\infty}$ values of test tablet and capsule over those of Herbesser-SR capsule was between 0.97 - 1.09 and 0.97 - 1.11. The acceptable bioequivalence range according to USP (Vol 1, 2007) is between 0.80 and 1.25. The confidence interval for all the three parameters (C_{max}, AUC_{0-t} and

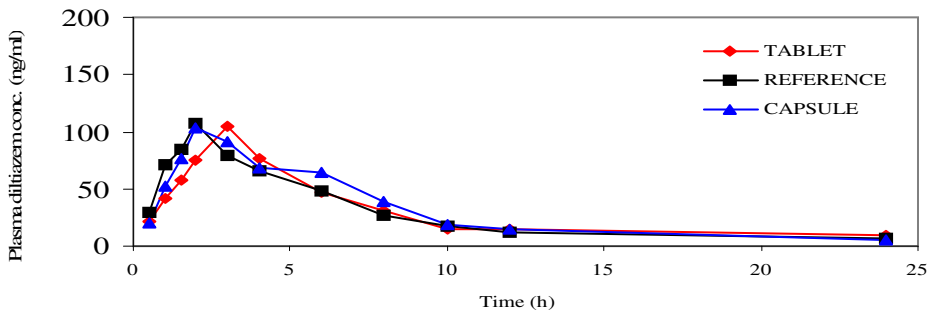

Figure 5. The *in vivo* pattern of all three formulations in subject-1.

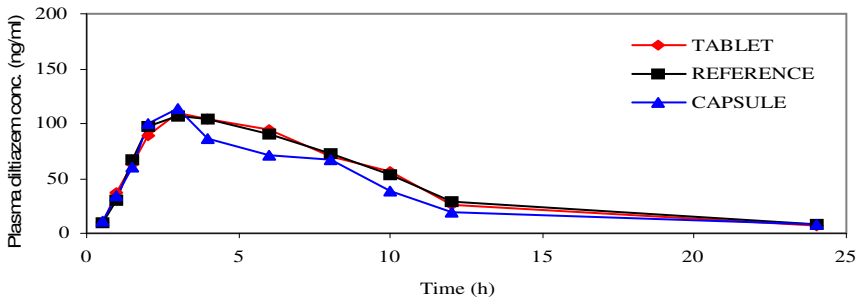

Figure 6. The in *vivo* pattern of all three formulations in subject-2.

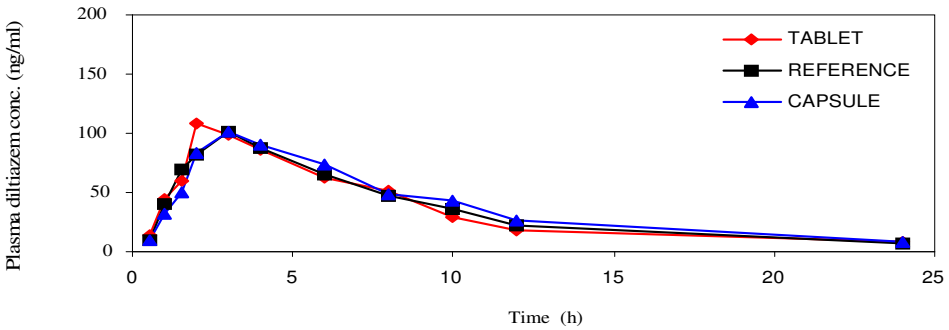

Figure 7. The *in vivo* pattern of all three formulations in subject-3.

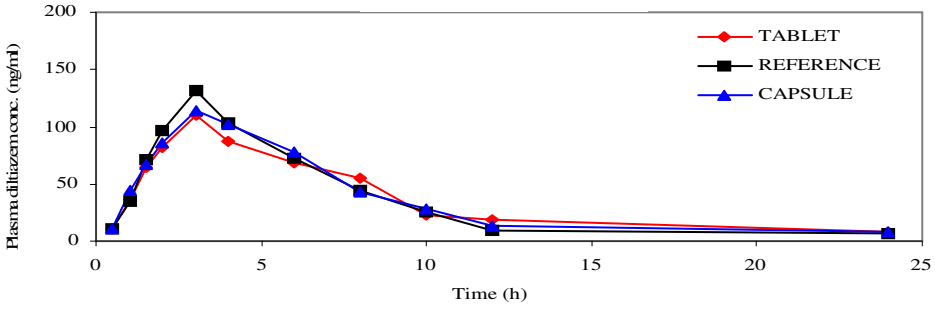

Figure 8. The *in vivo* pattern of all three formulations in subject-4.

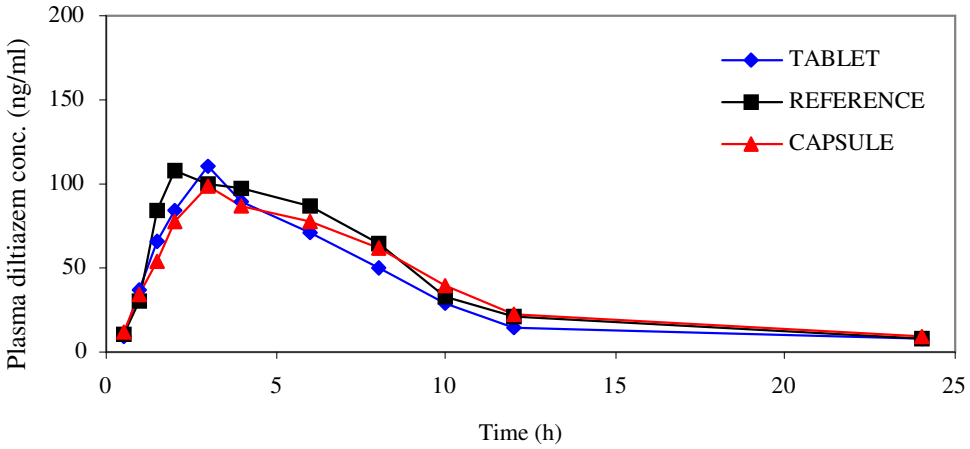

Figure 9. The *in vivo* pattern of all three formulations in subject-5.

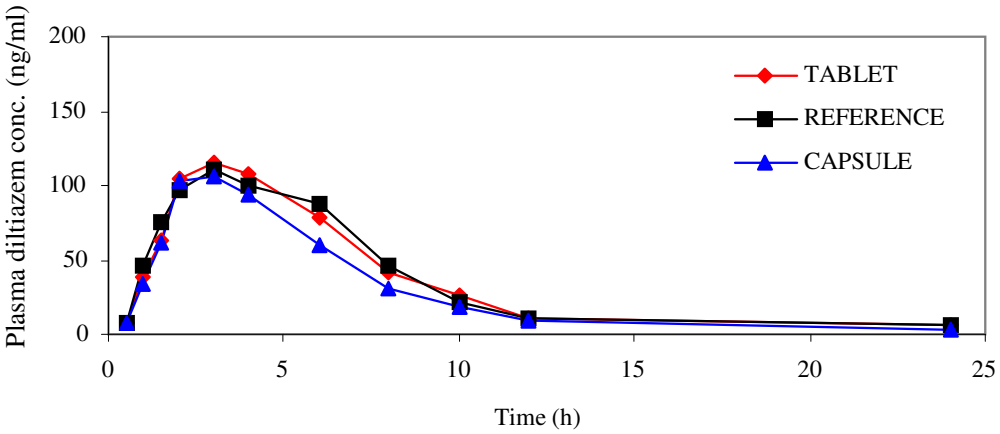

Figure 10. The *in vivo* pattern of all three formulations in subject-6.

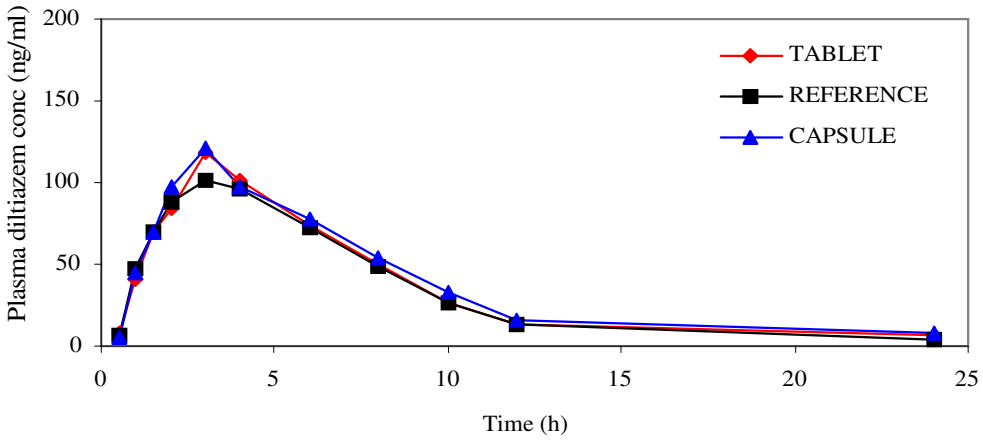

Figure 11. The *in vivo* pattern of all three formulations in subject-7.

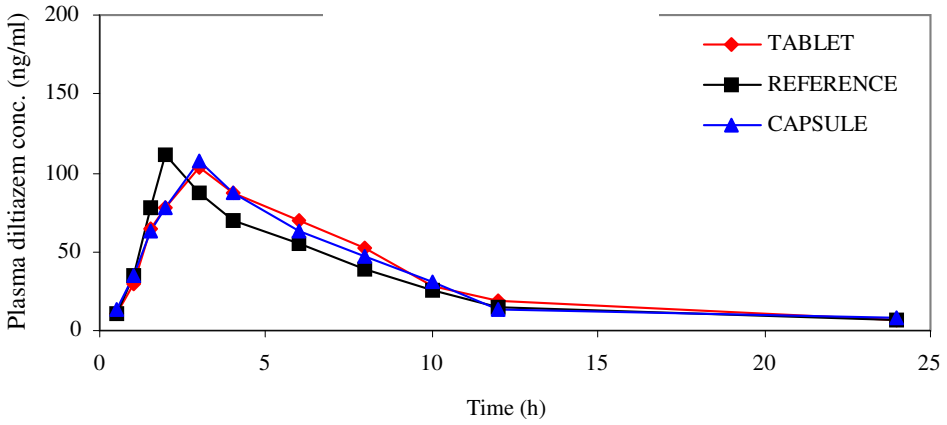

Figure 12. The *in vivo* pattern of all three formulations in subject-8.

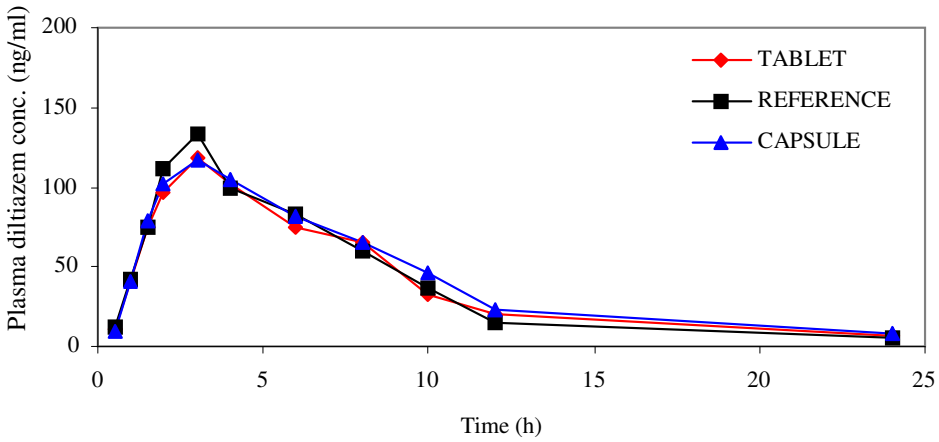

Figure 13. The *in vivo* pattern of all three formulations in subject-9.

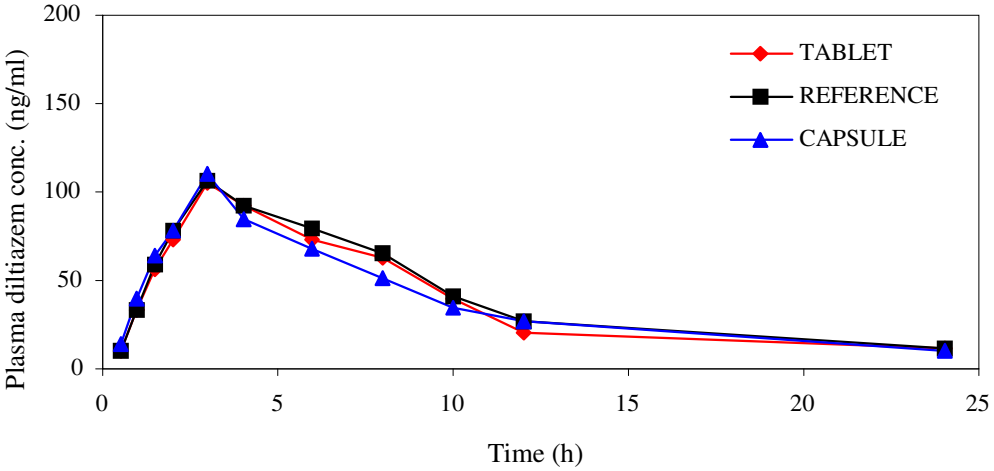

Figure 14. The *in vivo* pattern of all three formulations in subject-10.

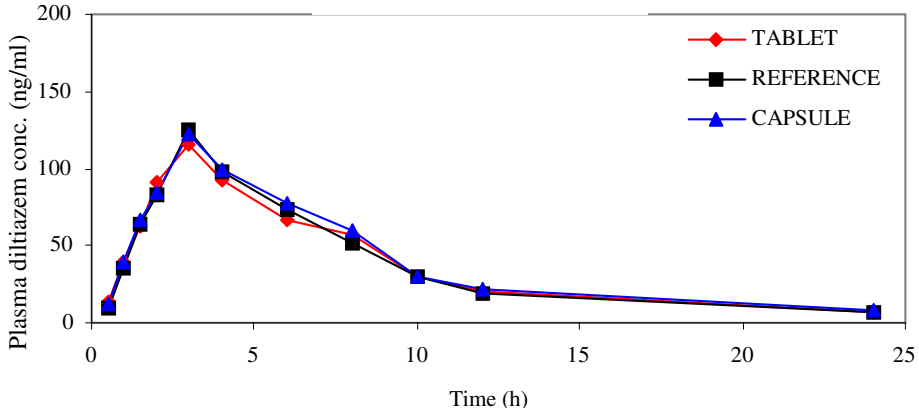

Figure 15. The *in vivo* pattern of all three formulations in subject-11.

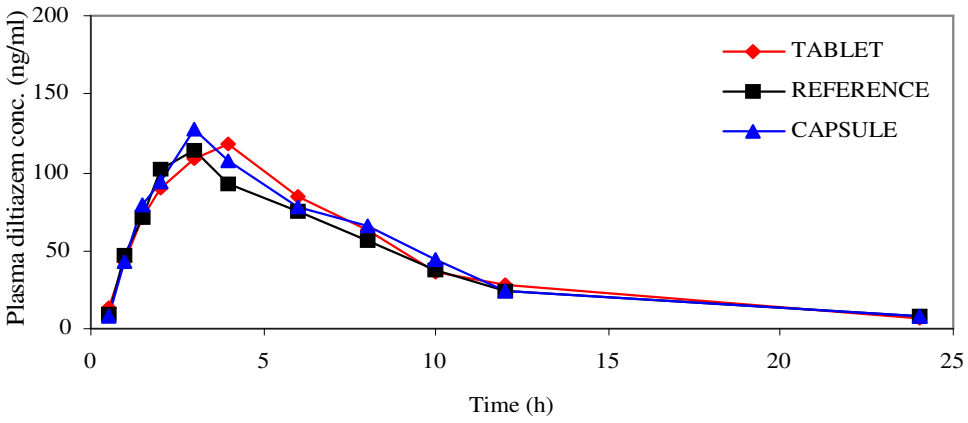

Figure 16. The in vivo pattern of all three formulations in subject-12.

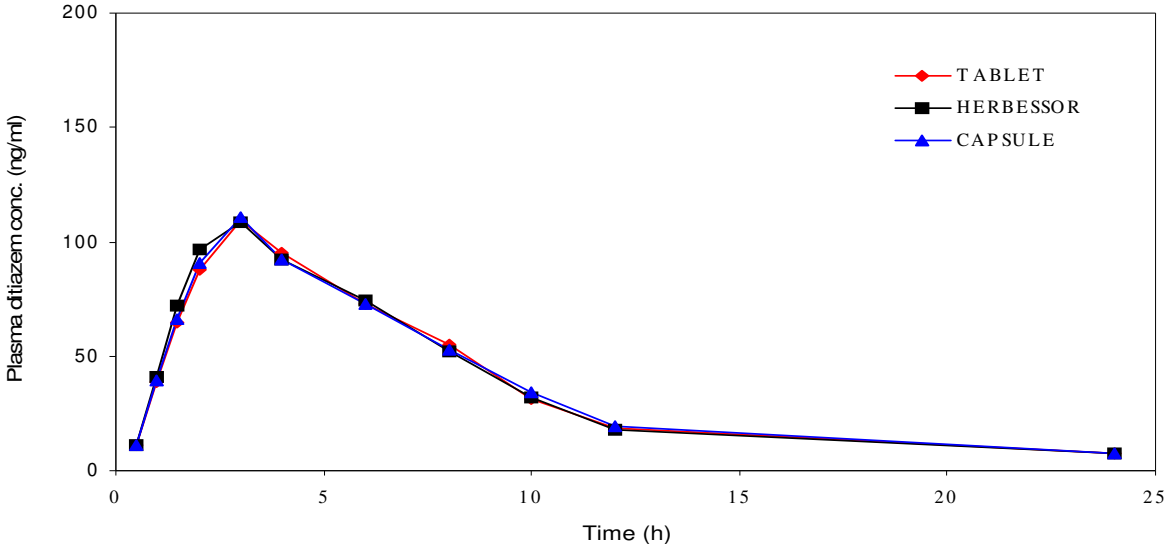

Figure 17. Mean plasma concentration of diltiazem from two test formulations and reference product (n = 12).

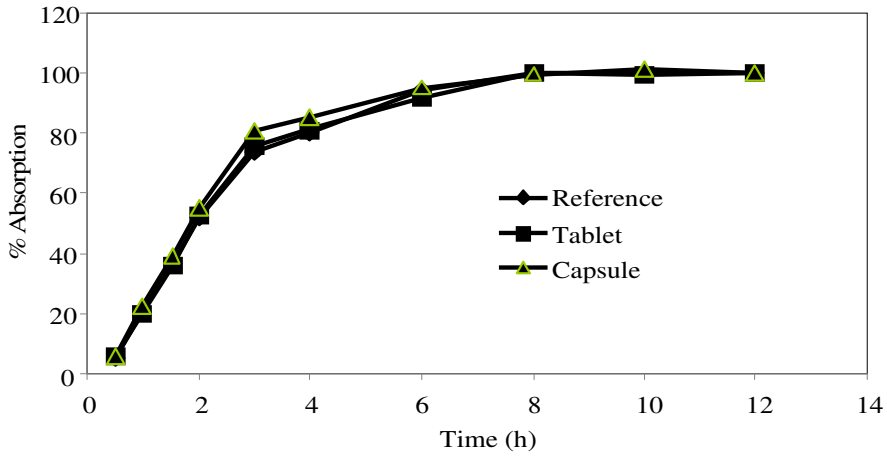

Figure 18. *In vivo* percent drug absorbed from two test formulations and reference product.

Table 7. Individual numerical values of AUC $_{0-\infty}$, AUC $_{0-t}$, C_{max}, T_{max}, Ke, $t_{1/2}$, Vd and MRT for reference.

Subject	AUC0-t	AUC0-∞	Cmax	Tmax	Ke	T1/2	Vd	MRT
S1	794.39	947.44	104.75	2.00	0.06	11.98	5.66	12.44
S2	1071.27	1135.23	106.84	3.00	0.13	5.44	8.72	8.90
S3	848.57	898.42	101.73	3.00	0.13	5.44	6.44	8.63
S4	875.53	929.59	112.81	3.00	0.12	5.57	6.67	8.44
S5	960.40	1045.99	107.18	2.00	0.06	11.98	5.48	12.42
S6	814.95	840.13	110.13	3.00	0.15	4.55	6.25	6.96
S7	813.23	840.24	101.50	3.00	0.15	4.59	7.00	7.34
S8	862.67	952.82	105.90	2.00	0.08	8.24	6.77	9.74
S9	1033.39	1071.69	133.35	3.00	0.15	4.70	6.59	7.65
S10	985.84	1080.50	106.92	3.00	0.11	6.40	7.45	10.16
S11	940.43	1018.06	120.60	3.00	0.10	7.08	6.61	9.22
S12	945.71	996.87	114.73	3.00	0.13	5.31	6.63	8.45
Mean	912.20	979.75	110.54	2.75	0.11	6.77	6.69	9.20
SD	90.77	94.76	9.03	0.45	0.03	2.66	0.83	1.77

Table 8. Individual numerical values of AUC $_{0-\infty}$, AUC $_{0-t}$, C_{max}, T_{max}, Ke, $t_{1/2}$, Vd and MRT for capsule.

Subject	AUC0-t	AUC0-∞	Cmax	Tmax	Ke	T1/2	Vd	MRT
S1	730.25	803.75	103.04	2.00	0.08	8.60	5.89	9.37
S2	905.99	958.71	113.65	3.00	0.13	5.40	7.16	8.56
S3	912.10	975.17	101.93	3.00	0.12	5.72	6.23	9.24
S4	838.89	880.42	114.56	3.00	0.13	5.17	5.78	7.93
S5	901.50	965.35	98.18	3.00	0.12	5.75	7.65	9.20
S6	680.49	695.92	106.62	3.00	0.16	4.20	4.99	6.41
S7	885.59	929.16	121.65	3.00	0.13	5.14	6.04	7.99
S8	841.17	931.33	100.48	3.00	0.08	8.24	6.85	9.94
S9	1006.90	1057.09	117.25	3.00	0.13	5.14	7.36	8.28
S10	912.57	1033.23	109.73	3.00	0.08	8.19	6.27	11.06
S11	930.59	1019.84	122.28	3.00	0.09	7.72	6.50	9.60
S12	1025.63	1089.85	127.86	3.00	0.13	5.33	6.91	8.55
Mean	880.97	944.98	111.43	2.92	0.12	6.22	6.47	8.84
SD	99.34	110.80	9.64	0.29	0.03	1.52	0.76	1.17

Table 9. Individual numerical values of AUC 0-∞, AUC 0-t, Cmax, Tmax, Ke, $t_{1/2}$, Vd and MRT for tablet.

Subject	AUC0-t	AUC0-∞	Cmax	Tmax	Ke	T1/2	Vd	MRT
S1	750.64	812.24	104.65	3.00	0.11	6.14	5.38	9.26
S2	1052.81	1095.70	108.99	3.00	0.15	4.76	8.74	8.23
S3	831.58	884.08	108.52	2.00	0.12	5.60	6.05	8.62
S4	829.61	948.01	109.67	3.00	0.07	9.94	6.67	11.08
S5	810.88	855.41	110.27	3.00	0.13	5.32	6.13	8.24
S6	817.86	847.74	114.73	3.00	0.15	4.75	5.60	7.23
S7	823.76	858.01	118.34	3.00	0.14	4.89	5.80	7.60
S8	817.50	890.98	103.20	3.00	0.09	7.40	6.74	9.40
S9	934.01	1000.96	118.75	3.00	0.10	6.79	6.98	8.73
S10	910.13	991.55	104.59	3.00	0.11	6.21	7.38	9.81
S11	873.60	932.68	115.36	3.00	0.11	6.51	6.43	8.64
S12	1025.63	1089.85	117.77	4.00	0.11	6.09	6.98	8.85
Mean	873.17	933.93	111.24	3.00	0.12	6.20	6.57	8.81
SD	91.31	93.90	5.61	0.43	0.02	1.44	0.91	1.02

Table. 10. Comparison of individual numerical values of AUC_{0-t} of test capsules and reference capsules.

	Reference capsules (R)	Test capsules (T)			
	AUC_{0-t}	AUC_{0-t}	T-R	T/R	ln (T/R)
S1	656.7	730.2	73.6	1.1	0.1
S2	1071.3	906.0	-165.3	0.8	-0.2
S3	848.6	912.1	63.5	1.1	0.1
S4	811.4	838.9	27.5	1.0	0.0
S5	960.4	901.5	-58.9	0.9	-0.1
S6	814.9	680.5	-134.5	0.8	-0.2
S7	788.7	885.6	96.9	1.1	0.1
S8	716.8	841.2	124.3	1.2	0.2
S9	925.2	1006.9	81.7	1.1	0.1
S10	985.8	912.6	-73.3	0.9	-0.1
S11	875.1	930.6	55.5	1.1	0.1
S12	945.7	1025.6	79.9	1.1	0.1
Mean	866.7	881.0	14.25	1.0	0.0
S.D	118.0	99.3	96.8	0.1	0.1

Table 11. Comparison of individual numerical values of AUC0-t of test tablets and reference capsules.

	Reference capsules (R)	Test tablets (T)			
	AUC_{0-t}	AUC_{0-t}	T-R	T/R	ln (T/R)
S1	656.7	750.64	94.0	1.14	0.13
S2	1071.3	1052.81	-18.5	0.98	-0.02
S3	848.6	831.58	-17.0	0.98	-0.02
S4	811.4	829.61	18.2	1.02	0.02
S5	960.4	810.88	-149.5	0.84	-0.17
S6	814.9	817.86	2.9	1.00	0.00
S7	788.7	823.76	35.1	1.04	0.04
S8	716.8	817.50	100.7	1.14	0.13
S9	925.2	934.01	8.8	1.01	0.01
S10	985.8	910.13	-75.7	0.92	-0.08
S11	875.1	873.60	-1.5	1.00	0.00

Table 11. Contd.

S12	945.7	1025.63	79.9	1.08	0.08
Mean	866.7	873.17	6.4	1.01	0.01
S.D	118.0	91.3	70.9	0.08	0.08

Table 12. Three way ANOVA table for Cmax using two test formulations and reference product.

Source	Type III Sum of Squares	Df	Mean square	F	Sig.
Subject	0.193	11	0.018	0.943	0.523
Period	0.023	2	0.011	0.610	0.553
Treatment	0.061	2	0.030	1.641	0.219
Error	0.371	20	0.019		
Corrected Total	0.648	35			

Table 13. Three way ANOVA Table for log transformed AUC 0-t using two test formulations and reference product.

Source	Type III Sum of squares	Df	Mean square	F	Sig.
Subject	1668.632	11	151.694	2.980	0.016
Period	1.800	2	0.900	0.018	0.982
Treatment	24.251	2	12.126	0.238	0.790
Error	1018.151	20	50.908		
Corrected Total	2712.833	35			

Table 14. Three way ANOVA table for log transformed AUC 0-∞ using two test formulations and reference product.

Source	Type III Sum of squares	df	Mean square	F	Sig.
Subject	0.379	11	0.034	6.400	0.000
Period	0.001	2	0.001	0.120	0.887
Treatment	0.001	2	0.000	0.056	0.946
Error	0.108	20	0.005		
Corrected Total	0.489	35			

Table 15. Three way ANOVA table for log transformed Cmax using two test formulations and reference product.

Source	Type III Sum of squares	Df	Mean square	F	Sig.
Subject	0.129	11	0.012	3.040	0.015
Period	0.000	2	0.000	0.021	0.979
Treatment	0.001	2	0.001	0.193	0.826
Error	0.077	20	0.004		
Corrected Total	0.208	35			

$AUC_{0-\infty}$) for both test capsule and tablet was with the given range Table 13, thus it can be inferred that both the test formulations were bioequivalent to the reference capsule (Herbesser SR).

A summary of the statistical analysis is given in Tables 12 - 15, which shows that the bioavailability and the extent of absorption between the three preparations were comparable. It is apparent from the tables that the sequence (or group) and period effect was not statistically significant suggesting that treatment-by-period interaction was insignificant.

The elimination rate constant, K_e of Herbesser-SR capsules and test capsules and tablets were 0.13 ± 0.03 and 0.12 ± 0.03 and 0.12 ± 0.02 h^{-1}, respectively and no statistical difference ($P > 0.05$) in K_e values of the three formulations were found. Similarly, the volume of distribution (V_d) of Herbesser-SR capsules, test capsules and tablets were 6.10 ± 1.21, 6.47 ± 0.76 and

Table 16. Confidence interval of test formulations compared to Reference for log transformed pharmacokinetic parameters.

	Capsule (Gelucire matrix)		Tablet (HPMC matrix)	
	Lower limit	**Upper limit**	**Lower limit**	**Upper limit**
AUC 0-t	0.91	1.08	0.96	1.06
AUC 0-∞	0.97	1.11	0.97	1.09
Cmax	0.94	1.04	0.94	1.03

Table 17. Correlation between *in-vitro* and *in-vivo* (IVIVC) data for the three formulations.

	Ref		Cap		Tab	
	in vitro	***in vivo***	***in vitro***	***in vivo***	***in vitro***	***in vivo***
20	1.11	0.91	1.41	0.92	1.32	0.99
40	2.77	1.47	3.93	1.56	3.83	1.67
60	5.99	2.06	7.5	2.15	7.65	2.3
80	11.74	3.9	11.98	3.88	12.06	3.93

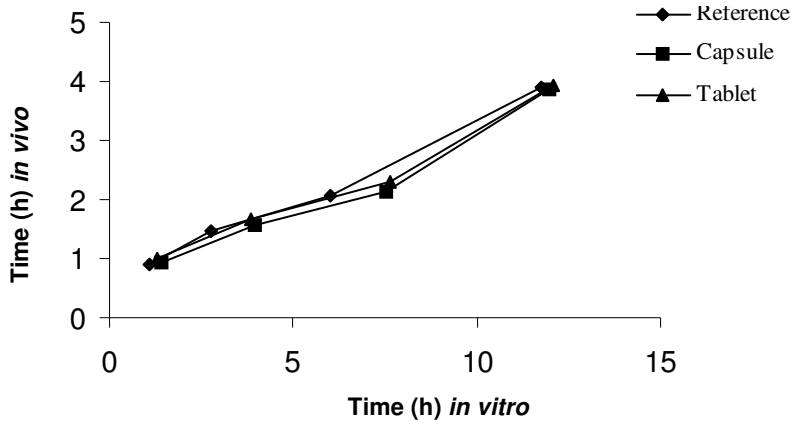

Figure 19. *In vitro* diltiazem HCl percent release versus *in vivo* absorption profiles from reference, test capsule and tablet.

6.57 ± 0.91 L/Kg and no statistical difference (P > 0.05) in V_d values of the three formulations were found. The elimination half-life ($t_{1/2}$) of Herbesser-SR capsules, test capsules and tablets were 5.69 ± 2.06, 6.22 ± 1.52 and 6.20 ± 1.44 h and the mean resident time (MRT) values were 8.42 ± 1.59, 8.84 ± 1.17 and 8.81 ± 1.02 h. No statistical difference was found in MRT and $t_{1/2}$ values of the test and reference formulations.

The plots of % *in vivo* absorption versus the % *in vitro* dissolution of the three formulations are shown in Figure 19. The plots appeared to be relatively linear. It is apparent that the three plots are divergent in nature. The value of correlation coefficients was calculated to be 0.9897 for reference, 0.9683 for capsule and 0.9732 for tablets. The slower rate of absorption was observed with test tablet compared to reference, followed by test capsule. In contrast, *in vitro* dissolution profiles indicated slower release rate in tablets followed by capsule and then reference. However, statistical correlation was observed between the *in vivo* absorption and *in vitro* dissolution data for three preparations as shown in Tables 14 and 17.

Conclusion

The results of *in vitro* drug release studies in water, 0.1 M HCl and pH 4 and 7 phosphate buffers showed that the solid matrix system and semi-solid matrix system were able to control the release of water soluble diltiazem hydrochloride. *In vivo* pharmacokinetic values of diltiazem hydrochloride obtained from tablet and capsule were comparable in the extent of bioavailability ($AUC_{0-\infty}$ and C_{max}) and in the rate of absorption ($C_{max\ and\ Tmax}$) and no

statistical differences were observed in log transformed data of AUC_{0-t} and C_{max} ($p > 0.05$) by comparing the three products on analysis of variance.

The 90% confidence interval for the ratio of log transformed $AUC_{0-\infty}$ values of the two test formulations was determined to be ranged between 0.97 - 1.09 and 0.97 - 1.11, being within the acceptable bioequivalence range of 0.80 to 1.25.Therefore, the *in vivo* pharmacokinetics evaluation of the two formulations showed a slow and prolonged release of diltiazem hydrochloride indicating the potential for clinical studies. Although all the three formulations are bioequivalent and statistically not different yet the lower C_{max}, prolonged MRT and $t_{½}$, and greater AUC mean values in twelve human volunteers of both test tablet and capsule compared to those of reference capsule indicated that the drug release from both test formulations is slow thereby providing a prolonged and controlled *in vivo* delivery of the drug. This proved the superiority of our test capsules and tablets over the reference capsules (Herbesser SR).

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