DISCUSSION
6.0. DISCUSSION

In the present study the Pharmacokinetic and Pharmacodynamic bioequivalence of three brands of diltiazem extended release capsules after single and multiple dose in healthy, male, adult, human subjects under fasting conditions was evaluated. The effect of drug administration on various Pharmacodynamic parameters after treadmill exercise was also studied.

The clinical study was carried out in accordance with ICH Good Clinical Practices. The study protocol and the informed consent form were approved by the Jamia Hamdard Institutional Review Board. Each of the subjects was required to understand and give his consent to participate in the study by signing the informed consent form. The signed original copy was retained.

The standard SOP’s of the clinical pharmacology unit and department of Metabolism and Pharmacokinetics (MAP) have been adhered to in the clinical, analytical, pharmacokinetic and statistical analysis.

Bioequivalence was assessed by measuring the:

1. Pharmacokinetic parameters namely $C_{\text{max}}$, $T_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$ for diltiazem
2. Pharmacodynamic parameter namely AUC$_{0-t}$ for diltiazem.

The study was conducted by using an open label, balanced, randomized, cross over design in healthy, male volunteers under fasting conditions (Annexure III). The order of receiving the test and reference products for each subject was determined according to a SAS generated randomization schedule (Annexure IV).

In this bioequivalence study, the reference product was Dilzem CD-120 extended release capsule, manufactured by Torrent Pharmaceuticals Ltd., India. The test products were Dilgard XL-120 extended release capsule (A), manufactured by Cipla Ltd., India; Angizem CD-120 (B) extended release capsule, manufactured by Sun Pharma Ltd, India.
The T/R ratios for log transformed data for the pharmacokinetic parameters $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were 98.16, 90.24 and 86.35 for test product A and were 109.49, 116.03 and 72.63 for test product B (Day 1) respectively. The T/R ratios for log transformed data for the pharmacokinetic parameters $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were 108.13, 89.09 and 86.14 for test product A and were 108.91, 99.24 and 91.58 for test product B (Day 7) respectively. The USFDA (CDER, 2003) guidelines recommend that the T/R ratios of log transformed data should be as close as possible to 95-105% range. The T/R ratio for $AUC_{0-t}$ and $AUC_{0-\infty}$ for test product A (Day 1) was out of range. The T/R ratios for Test product B fell outside the acceptable limits for all the pharmacokinetic parameters. The T/R ratio for test product A (Day 7) was also out of range for all the pharmacokinetic parameters. The T/R ratio for test product B (Day 7) was also out of range for all the pharmacokinetic parameters except $AUC_{0-t}$.

However, when 90% confidence interval was applied for log transformed data, the 90% Confidence Intervals for $AUC_{0-t}$ and $AUC_{0-\infty}$ (except $C_{\text{max}}$) fell outside the 80-125% range for product A (Day 1). The 90% confidence interval for product B (Day 1) was out of range for all the pharmacokinetic parameters. The 90% confidence interval for both the test products A & B (Day 7) fell outside the prescribed bioequivalence limit of 80-125% for all the pharmacokinetics parameters (except $AUC_{0-t}$ for product B).

Hence, after single dose (Day 1), the overall statistical results for both the comparisons (i.e. A vs R and B vs R) did not indicate bioequivalence or potential bioequivalence.

After multiple dosing also, the confidence intervals were mostly outside the prescribed limit (80-125%) for both the test products for all the 3 pharmacokinetic parameters (except for parameter $AUC_{0-t}$ for comparison B vs R), hence both the test products were not bioequivalent with the reference product on Day 7.

The Intra subject variability for the pharmacokinetic parameters $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ was very high for Day 1. With such high intra subject variability the estimated confidence interval becomes so wide and it is very difficult to remain within preset bioequivalence limits even if the geometric means ratio is an ideal 100%.
(Midha et al, 1993) and it requires a larger sample size to conclude bioequivalence and as a result bioequivalence study becomes very expensive and cumbersome. For example two formulations with greater than 30% intra subject CV would require a 52-subject study to have a high chance (90%) of meeting the acceptance criteria, assuming a true relative bioavailability of 1.05 (Blume et al, 1992). The p-values (Day 1) were not significant for the effects studied i.e. there was no treatment, period and sequence effect observed (p-values for these effects > 0.05 from the ANOVA model). Some of the important findings in this study were high intra subject variability observed for the pharmacokinetic parameters and the variability observed between subject profiles. The power of the test was low and for $\text{AUC}_0-\infty$ it was significantly low (Day 1).

The intra subject variability was significantly low for Day 7, ranging between 20-26% for all the parameters, indicating low variability in the data.

The power of the statistical test was high, ranging between 63-82%, indicating the probability of detecting bioequivalence was high.

There were no significant treatment, period or sequence effects (p-values for these effects > 0.05 from the ANOVA model)

Based on these results we can say that although the confidence intervals were mostly outside the limits for both the comparisons for all the 3 pharmacokinetic parameters on Day 7 (except for parameter $\text{AUC}_0-t$ for B), still A and B could be said to potentially bioequivalent based on the overall results, and the results indicated the bioequivalence could have been proved on a larger number of subjects.

**Product Quality**

If we compare the $C_{\text{min}}$ and $C_{\text{max}}$ values at different days then we can get a good idea about the quality and drug release behavior of various products.

**STEADY STATE SCATTER**

**Steady state $C_{\text{min}}$ value on Day 7**

Comparing the steady state $C_{\text{min}}$ values on Day 7, we find that it is highest with product R (53.80 ng/ml), for product A, it is 35.3% less than R and for product B it is 41.44% less than R (Table PK8). Thus $C_{\text{min}}$ concentrations at SS were in the order $R > A > B$. Here also product R is faring better than both the test products.
Steady state C_{min} value on Day 8
Comparing the steady state C_{min} values on Day 8, we find that it is highest with product R (50.39 ng/ml), for product A it is 24.48% less than R and for product B it is 39.88% less than R (Table PK8).
Thus, steady state variability in C_{min} is least with product R, for B it is nearly same as R but product A shows marked variation. Based on the steady state C_{min} values on Day 7 and Day 8 we can conclude that product A is more variable than product R and B.

Ratio of C_{min} values on Day 7 and Day 8
On comparing the ratio of C_{min} values on Day 7 and Day 8, we find that for product R, the ratio is 1.06 i.e. very near to the acceptable limit of 0.95-1.05. The ratio for product A (0.91) is outside the acceptable limit while for product B (1.03) it is within acceptable limit (Table PK9).

Steady state C_{max} value on Day 7
Comparing the steady state C_{max} values on Day 7, we find that it is highest with product A (89.20 ng/ml) and lowest for product R. For product A it is 10.61% more than R and for product B it is 7.40% more than R (Table PK10). Hence product A is more variable than product B, when compared with reference.

Comparison of percentage increase in C_{max} on Day 7
Product R has an increase of 44.77% in C_{max} on Day 7 over Day 1.
Product A has an increase of 62.56% in C_{max} on Day 7 over Day 1
Product B has an increase of 49.89% in C_{max} on Day 7 over Day 1 (Table PK10)
Ideally a SR product shall not exhibit high C_{max} peak under steady state conditions, as it can precipitate concentration dependent adverse drug reactions.
On this basis, we can conclude that product B is quite comparable to that of product R while product A is highly variable.

Increase in plasma concentrations after drug administration on Day 7
On comparing the increase in plasma concentrations on Day 7 (after multiple doses of diltiazem for 7 days), we find that product A and B produced a greater than 12.91%
and 50.26% respectively, swing in plasma concentrations as compared to reference compared (Table PK 11). Hence neither of the test products was behaving identically to the reference product.

Comparison of AUC on Day 1 and Day 7
On comparing the AUC values for different products on different days, it is clearly visible that AUC values of product B were quite close to those of product R while for product A, the AUC values were quite different (Table PK12). Hence we can conclude that at steady state (Day 7), both the test products A and B were not comparable to reference product R for AUC0-36, as well as for AUC12-24 hrs.

Peak: Trough ratios at steady state
The peak to trough ratios for SR preparations must be maintained above 50% i.e. higher the ratio, the ‘better’ it is (Palatini et al, 2002). In our study only reference (R) product exhibited a peak: trough ratio of 62.48% at 24 hrs after administration of the last dose. None of the products tested met this goal 36 hrs after administration of the last dose (Table PK13). This has therapeutic implications, if a patient on diltiazem SR happens to miss a dose. Very low (undetectable) plasma concentrations shall remain between 36-48 hrs after the last dose with all the three formulations (Table PK13 & Table PK15). Therefore, serious attention must be paid to ensure patient’s compliance to treatment especially when SR formulations are prescribed.

Time to reach P/T ratio of 50% at steady state
Time to reach 50% of the peak concentration for different products is shown in Table PK14. It can be seen that reference (R) product takes 14 hrs to reach to 50% of the peak concentration and it occurs at 26 hrs after drug administration. Product A takes 7 hrs to reach to 50% of the peak concentration and it occurs at 9 hrs after drug administration. Product B takes 13 hrs to reach to 50% of the peak concentration and it occurs at 20 hrs after drug administration. Thus, product A and B failed to maintain desirable plasma concentrations for 15 and 4 hours respectively before the next dose becomes due.
Decrease in $C_{\text{min}}$ values at 36 hrs and 48 hrs (hypothetical)

The decrease in plasma concentration ($C_{\text{min}}$) at 36 hrs with reference to 24 hrs levels was appx. 63% with all the three formulations (Table PK15). This indicates that the differences in the absorption parameters observed were due to the formulation factors. Drug, diltiazem is behaving identically in all the formulations, but the difference lies in the way these formulations were prepared. Different manufacturers use different formulation techniques to prepare SR products to get desired release profile and hence these differences in absorption are seen.

Table PK15: Decrease in $C_{\text{min}}$ values at 36 hrs and 48 hrs (hypothetical) with reference to $C_{\text{min}}$ value at 24 hrs

<table>
<thead>
<tr>
<th>Product</th>
<th>$C_{\text{min}}$ Day 8 (24hrs) (ng/ml)</th>
<th>$C_{\text{min}}$ Day 8 (36hrs) (ng/ml)</th>
<th>%age reduction</th>
<th>$C_{\text{min}}^*$ Day 9 (48hrs) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>50.39</td>
<td>18.36</td>
<td>63.56 %</td>
<td>11.56</td>
</tr>
<tr>
<td>A</td>
<td>38.05</td>
<td>13.11</td>
<td>65.54 %</td>
<td>8.25**</td>
</tr>
<tr>
<td>B</td>
<td>30.29</td>
<td>11.06</td>
<td>63.48 %</td>
<td>6.96**</td>
</tr>
</tbody>
</table>

* 63% reduction preserved  
** Undetectable levels  
Ω Missed dose on Day 8 presumed

Table PK16: Hypothetical $C_{\text{min}}$ and $C_{\text{max}}$ values of diltiazem on Day 7 after multiple dosing with diltiazem 240 mg diltiazem ER formulation

<table>
<thead>
<tr>
<th>Product</th>
<th>$C_{\text{min}}$ at SS (Day 7) (ng/ml)</th>
<th>$C_{\text{max}}$ at SS (Day 7) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>107.6</td>
<td>161.2</td>
</tr>
<tr>
<td>A</td>
<td>69.6</td>
<td>178.4</td>
</tr>
<tr>
<td>B</td>
<td>63.0</td>
<td>173.2</td>
</tr>
</tbody>
</table>
Hypothetical $C_{\text{min}}$ and $C_{\text{max}}$ values of diltiazem 240 mg SR formulation

It is reported that slow release preparations of diltiazem exhibits linear kinetics (Mould, 2002). Hence assuming linear kinetics, if we determine the hypothetical $C_{\text{min}}$ and $C_{\text{max}}$ values on Day 7 (multiple dosing) for 240 mg diltiazem ER formulation then the $C_{\text{max}}$ values are above the negative chronotropic concentrations i.e. 150 ng/ml (Dash et al., 1985) (Table PK16).

In our study (120 mg dose), all concentrations were below 150ng/ml. this could be the reason for not getting any clinically significant changes in the pharmacodynamic parameters.

Characteristics of an ideal sustained release (SR) formulation,

Some of the characteristics of an ideal sustained release formulation at steady state are:

1. Limited fluctuations within therapeutic range (increased safety and efficacy) because SR preparations are not intended for use in acute conditions but are used only in maintenance therapy

2. Avoidance of undesirable high peaks (toxicity) of blood levels after administration of each dose (Least scatter in $C_{\text{max}}$) to avoid concentration dependent ADE’s.

3. A peak to trough ratio of at least 50% until the next dose is due.

4. Avoidance of undesirable low troughs (sub therapeutic) of blood levels (Least scatter in $C_{\text{min}}$)

Product R fulfills all of these criteria.

Product A shows marked variation in the SS concentrations on Day 7 and Day 8. In case of product A, $T_{\text{max}}$ values on Day 1 and Day 7 are very less (Day 1 $T_{\text{max}}$ is 1-2 hrs for 10/17 subjects and 10-24 hrs for 7 /17 subjects, while on Day 7- $T_{\text{max}}$ is 1-2 hrs for 16/17 subjects and 14 hrs for only 1/17 subjects).

For products R and B, the $T_{\text{max}}$ values are in the range of 8-24 hrs and 6-14 hrs respectively for majority of the subjects (Table PK2, PK3 & PK4).
This data suggests that in case of product A ($T_{max} = 1.94$ hrs on Day 7) dose dumping is occurring on Day 7. This may lead to toxic effects and failure to maintain effective therapeutic concentration over 24 hr period in patients.

Based on above discussion we can conclude that product R fulfils the characteristics of an ideal SR preparation. Product A does not fulfill these characteristics, while product B fulfills only some of these characteristics. Thus neither product A nor product B can be safely and confidently substituted for product R. Once a patient is stabilized on a particular SR formulation of diltiazem, the physician must avoid shifting the patient to another SR formulation without pressing and valid therapeutic reasoning.

In this study we had originally planned to use 240 mg dose of diltiazem but later used 120 mg dose because of ethical constraints. This was a multiple dose study and was designed on an out patient basis, except on sampling days when the volunteers stayed in house. It was recommended by the Jamia Hamdard Institutional Review Board (JHIRB) to reduce the dose from 240 mg to 120 mg on safety grounds as it was only a bioequivalence study and not an efficacy study.

Moreover in an earlier bioavailability study conducted at Ranbaxy Clinical Pharmacology Unit in which on multiple doses of 240 mg diltiazem once daily formulation were administered to healthy human volunteers, lot of adverse events were reported (2 subjects developed 2° heart-block; 2 subjects complained of chest pain which could not be substantiated; 1 subject reported of postural hypotension; 2 subjects experienced headache and felt claustrophobic, all these subjects were withdrawn from the study).

It is reported in literature that plasma concentrations of approximately 150ng/ml produce negative chronotropic effects (Dash et al, 1985). It has been proved that slow release preparations of diltiazem exhibits linear kinetics (Mould, 2002). Hence assuming linear kinetics, if we determine the hypothetical $C_{min}$ and $C_{max}$ values on Day 7 (multiple dosing) for 240 mg diltiazem ER formulation then the $C_{max}$ values are above the negative chronotropic concentrations (150 ng/ml). The results are shown in Table PK16.
There are many studies reported in literature on the multiple dose pharmacokinetics of 240 mg diltiazem. An earlier study in literature reported the $C_{\text{max}}$ and $C_{\text{min}}$ values after multiple dosing of 240 mg diltiazem ER formulation in healthy subjects as 156.3 ng/ml and 48 ng/ml respectively (Kelly et al, 1991).

Smith et al (1995) reported that after administration of 240 mg diltiazem ER preparations for 6 days in healthy human subjects, the $C_{\text{max}}$ and $C_{\text{min}}$ values obtained were 180.6 ng/ml and 49.9 ng/ml respectively. In another study conducted by the same authors, the $C_{\text{max}}$ and $C_{\text{min}}$ values after multiple dosing of 240 mg diltiazem ER formulation in healthy subjects were reported to be 135.6 ng/ml and 28.9 ng/ml respectively (Smith et al, 1995).

In a study conducted at Ranbaxy Clinical Pharmacology Unit (1996) the $C_{\text{max}}$ and $AUC_{0-t}$ obtained after administration of single dose of Dilacor XR (Rhone-Poulenc Rorer, USA) 240 mg diltiazem capsules were 205.65 ng/ml and 4252.16 ng.hr.ml$^{-1}$ (Unpublished data). This concentration was much above the concentration at which negative chronotropy occurs (150 ng/ml) (Dash et al, 1985).

In yet another study, the $C_{\text{max}}$ and $C_{\text{min}}$ values after multiple dosing with 240 mg diltiazem in healthy subjects were found to be 178 ng/ml and 44 ng/ml respectively (Eradiri & Midha, 1997). Hence in most of the multiple dose studies with 240 mg diltiazem, the $C_{\text{max}}$ values exceeded 150 ng/ml (concentration above which negative chronotropy occurs). Thus on this basis we selected a lower dose of diltiazem (120 mg).

There are many other bioequivalent studies on different doses of diltiazem SR reported in literature.

Ruio et al (1990) studied bioequivalence of two different pharmaceutical forms of diltiazem. The preparations were concluded to be bioequivalent but again the confidence interval, acid test for determining bioequivalence, was not employed.

In another study (Christolup et al, 1992) single dose and steady state pharmacokinetics of two marketed controlled release diltiazem 120 mg formulations (Cardil, Denmark and Cardizem, Denmark) was studied. Following single dose administration, a significant difference in $T_{\text{max}}$ was seen (2.9±1.9 and 6.8±2.6 hrs respectively) whereas
differences in AUC, $t_{1/2}$ and $C_{\text{max}}$ were not significant. The AUC values following single dose administration of Cardil and Cardizem were 678.4±321.5 and 948.6±580.6 ng. hr /ml respectively. At steady state $T_{\text{max}}$ being (2.7±2.0 and 6.0±2.8 hrs respectively). The AUC values at steady state being 880.1±399.8 and 1056.8±509.8 ng. hr /ml respectively. The products were concluded to be bioequivalent based on 95% confidence intervals. In our study the $T_{\text{max}}$ after single dose were 11.88 (+6.49), 7.70 (+8.73) and 8.47 (+2.06) hrs for products R, A and B respectively. We see that $T_{\text{max}}$ values are quite higher in our formulations. The $AUC_{0-\infty}$ following single dose were 807.75 (+397.86), 714.44 (+252.47) and 894.72 (+366.41) ng.hr/ml for products R, A and B respectively. These are quite close to those reported by earlier workers.

Dange et al., (1992) studied the comparative bioavailability of two 60 mg formulations of diltiazem (immediate release, IR vs modified release, MR) in 8 healthy volunteers was studied. After administration of single dose of test formulation (MR) formulation, the $C_{\text{max}}$ obtained was 67.50± 9.54 ng/ml at 3.75± 0.43 hrs while that of the reference formulation (IR) was 120.50± 21.90 at 0.58± 0.30 hrs. The $AUC_{0-\infty}$ was found to be 500.25± 87.56 ng.hr/ml and 483.65±91.70 ng.hr/ml for reference and test formulations respectively. It was concluded in this study that MR formulation had better PK profile as compared to IR formulation. But confidence intervals were not taken into account which is a crucial requirement as per the DCGI as well as US FDA (CDER, 2003) to conclude bioequivalence.

Guimont et al., (1993) studied comparative pharmacokinetics of single dose of two marketed sustained release formulations of diltiazem 120 mg. The $C_{\text{max}}$ of product A and B were found to be 89.1±30.3 ng/ml and 61.1 ng/ml respectively. The $T_{\text{max}}$ were found to be 6.8±1.0 and 6.1±1.0 hrs for product A and B respectively. It was concluded that the products were not bioequivalent based on 90% confidence intervals for $C_{\text{max}}$ and $AUC_{0-\infty}$. The results obtained in our study are also in agreement with those of Guimont et al., but in our study, one of the test products (B) was bioequivalent with the reference after single dose.
Lippert et al., (1996) studied relative bioavailability of Dilaclor XR capsules compared to Cardizem CD capsules at 180 mg and 540 mg dose levels. It was concluded that both the products were not bioequivalent at either of the dose levels. Here also confidence intervals were not used to prove bioinequivalence.

In few other studies (Eradiri & Midha, 1997; Luckow & Paschoa, 1997; Quiroga et al, 2001) reported in literature different strengths of diltiazem were used and confidence intervals were utilized to prove bioequivalence.

On comparing the pharmacodynamic bioequivalence using various pharmacodynamic parameters (i) PR-interval- An increase as well as decrease in PR-interval at different time points was seen after treatment with different formulations of diltiazem. The reason may be that the dose used may not be sufficient to elicit a pharmacodynamic response in healthy volunteers. The 90% CI of none of the formulations (Day1 & Day7) were within the prescribed limit of bioequivalence. Hence none of the test products were bioequivalent to the reference product.

Pharmacokinetie-Pharmacodynamic (PK-PD) correlation
The reference (R) formulation produced maximum prolongation of PR-interval at 16 hrs on Day1 and the T\textsubscript{max} of this formulation was also found at 17.76 hrs. On Day 7, maximum prolongation was of PR-interval was produced at 10 hrs and the T\textsubscript{max} was also found at 11.88 hrs. The test product A produced maximum prolongation of PR-interval at 2 hrs on Day1 and the T\textsubscript{max} of this formulation was found at 7.7 hrs. On Day 7, maximum prolongation was of PR-interval was produced at 2 hrs and the T\textsubscript{max} was also found at 1.94 hrs. The test product B produced maximum prolongation of PR-interval at 8 hrs on Day1 and the T\textsubscript{max} of this formulation was found at 8.47 hrs. On Day 7, maximum prolongation was of PR-interval was produced at 6 hrs and the T\textsubscript{max} was also found at 6.82 hrs. Thus we can correlate the PK effect with the PD effect.

(ii) QTc-interval- An increase as well as decrease in QTc-interval at different time points was seen after treatment with different formulations of diltiazem. The reason may be that the dose used may not be sufficient to elicit a pharmacodynamic response
in healthy volunteers. The 90% CI of none of the formulations (Day 1 & Day 7) were within the prescribed limit of bioequivalence. Hence none of the test products were bioequivalent to the reference product.

**Pharmacokinetic-Pharmacodynamic (PK-PD) correlation**

The reference (R) formulation produced maximum prolongation of QTc -interval at 16 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was also found at 17.76 hrs. On Day 7, maximum prolongation was of QTc -interval was produced at 16 hrs and the $T_{\text{max}}$ was found at 11.88 hrs. The test product A produced maximum prolongation of QTc -interval at 8 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was found at 7.7 hrs. On Day 7, maximum prolongation was of QTc -interval was produced at 8 hrs and the $T_{\text{max}}$ was found at 1.94 hrs. The test product B produced maximum prolongation of QTc -interval at 16 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was found at 8.47 hrs. On Day 7, maximum prolongation was of QTc -interval was produced at 6 hrs and the $T_{\text{max}}$ was also found at 6.82 hrs. Thus we can correlate the PK effect with the PD effect.

(iii) HR- An increase as well as decrease in HR at different time points was seen after treatment with different formulations of diltiazem. The reason may be that the dose used may not be sufficient to elicit a pharmacodynamic response in healthy volunteers. The 90% CI of none of the formulations (Day 1 & Day 7) were within the prescribed limit of bioequivalence. Hence none of the test products were bioequivalent to the reference product.

**Pharmacokinetic-Pharmacodynamic (PK-PD) correlation**

The reference (R) formulation produced maximum reduction in HR at 24 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was found at 17.76 hrs. On Day 7, maximum reduction was of HR was produced at 2 hrs and the $T_{\text{max}}$ was found at 11.88 hrs. The test product A produced maximum prolongation of HR at 24 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was found at 7.7 hrs. On Day 7, maximum reduction was of HR was produced at 26 hrs and the $T_{\text{max}}$ was found at 1.94 hrs. The test product B produced maximum reduction of HR at 24 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was found at 8.47 hrs. On Day 7, maximum reduction was of HR was
produced at 28 hrs and the $T_{\text{max}}$ was found at 6.82 hrs. Thus here we cannot correlate the PK effect with the PD effect.

(iv) SBP- An increase as well as decrease in SBP at different time points was seen after treatment with different formulations of diltiazem. The reason may be that the dose used may not be sufficient to elicit a pharmacodynamic response in healthy volunteers. The 90% CI of none of the formulations (Day1 & Day7) were within the prescribed limit of bioequivalence. Hence none of the test products were bioequivalent to the reference product.

Pharmacokinetic-Pharmacodynamic (PK-PD) correlation

The reference (R) formulation produced maximum reduction in SBP at 24 hrs on Day1 and the $T_{\text{max}}$ of this formulation was found at 17.76 hrs. On Day 7, maximum reduction was of SBP was produced at 26 hrs and the $T_{\text{max}}$ was found at 11.88 hrs. The test product A produced maximum prolongation of SBP at 24 hrs on Day1 and the $T_{\text{max}}$ of this formulation was found at 7.7 hrs. On Day 7, maximum reduction was of SBP was produced at 2 hrs and the $T_{\text{max}}$ was found at 1.94 hrs. The test product B produced maximum reduction of SBP at 24 hrs on Day1 and the $T_{\text{max}}$ of this formulation was found at 8.47 hrs. On Day 7, maximum reduction was of SBP was produced at 28 hrs and the $T_{\text{max}}$ was found at 6.82 hrs. Thus here we cannot correlate the PK effect with the PD effect completely.

(v) DBP- An increase as well as decrease in DBP at different time points was seen after treatment with different formulations of diltiazem. The reason may be that the dose used may not be sufficient to elicit a pharmacodynamic response in healthy volunteers. The 90% CI of none of the formulations (Day1 & Day7) were within the prescribed limit of bioequivalence. Hence none of the test products were bioequivalent to the reference product.

Pharmacokinetic-Pharmacodynamic (PK-PD) correlation

The reference (R) formulation produced maximum reduction in DBP at 24 hrs on Day1 and the $T_{\text{max}}$ of this formulation was found at 17.76 hrs. On Day 7, maximum reduction was of DBP was produced at 36 hrs and the $T_{\text{max}}$ was found at 11.88 hrs. The test product A produced maximum prolongation of DBP at 16 hrs on Day1 and the $T_{\text{max}}$ of this formulation was found at 7.7 hrs. On Day 7, maximum reduction was of DBP was produced at 16 hrs and the $T_{\text{max}}$ was found at 1.94 hrs. The test product B
produced maximum reduction of DBP at 10 hrs on Day 1 and the $T_{\text{max}}$ of this formulation was found at 8.47 hrs. On Day 7, maximum reduction was of DBP was produced at 36 hrs and the $T_{\text{max}}$ was found at 6.82 hrs. Thus here we cannot correlate the PK effect with the PD effect completely.

Rate-pressure product (RPP)

RPP is a non-invasive index of myocardial oxygen demand. The RPP on Day 1 before exercise (at rest) increased at 6 hrs for products R and A, while for product B it decreased at 6 hrs after administration of diltiazem at rest. At 12 hrs on Day 1, all the products decreased RPP at rest after administration of diltiazem.

After exercise (120 seconds of exercise on treadmill) the RPP was increased at all time points for all the products.

The RPP on Day 7 before exercise (at rest) increased at 6 hrs, 12 hrs, 30 hrs and 36 hrs, while it decreased at 24 hrs for all products after administration of diltiazem.

After exercise the RPP was increased at all time points for all the products. The minimum decrease in RPP on Day 7 was seen at 12 hrs for all the products.

There are reports in literature about the decrease in RPP at rest during administration of diltiazem 180 to 360 mg and during submaximal exercise in patients receiving 360 mg daily (Go & Hollenberg, 1984). The reductions in RPP suggest that diltiazem enhances exercise time and lengthens time to onset of myocardial ischaemia by decreasing myocardial oxygen demand. In our study, on Day 1, all the products decreased RPP at 12 hrs at rest after administration of diltiazem. After exercise, RPP was increased at all time points for all the products but minimum decrease in RPP on Day 7 was seen at 12 hrs for all the products. Hence diltiazem was most effective in decreasing myocardial oxygen demand at 12 hrs.

There are number of reports in literature where pharmacodynamic bioequivalence of diltiazem has been studied.

Dange et al., (1992) studied the comparative pharmacodynamics of two 60 mg formulations of diltiazem (immediate release, IR vs modified release, MR) in 8 healthy volunteers. Pharmacodynamic parameters like SBP, DBP, sinus rate and PR-
intervals were monitored during the 12 hr study. Administration of MR formulation caused significantly (p<0.05) less alterations in PD parameters. The 90% CI approach was not used in this study and it was concluded that further studies would be necessary in patients to confirm these findings. These results are also in agreement to our study where no clinically significant PD effects were seen.

Guimont et al., (1993) studied comparative pharmacodynamics of two marketed sustained release formulations of diltiazem 120 mg. Pharmacodynamic parameters like SBP, DBP, sinus rate and PR-intervals were studied. The only pharmacodynamic parameters showing statistically significant difference in change from baseline between the formulations were DBP (p=0.0135) and PR-intervals. But the changes from baseline for these pharmacodynamic parameters were not clinically relevant for either formulation. In our study also there are no clinically significant changes in any of the pharmacodynamic parameters. In our study, none of the test products were pharmacodynamically bioequivalent as the 90% confidence intervals were not within prescribed limits. The reason for this weak clinical response of diltiazem could be (i) volunteers were ‘healthy’, with a low blood pressure at entry (ii) Also, it is well known that calcium channel blockers (Akoki et al, 1983; MacGregor et al, 1982) and antihypertensive agents (Gill et al, 1985; Sumner et al, 1988) lower blood pressure to a greater extent in hypertensive patients than in normotensive subjects. Our findings are in agreement with previous studies in healthy subjects where no significant effect was observed in SBP (Akoki et al, 1983; Boyd et al, 1989) and sinus rate (Akoki et al, 1983; Kinney et al, 1981) after oral diltiazem administration.

The results obtained in our study are in agreement with Guimont et al, 1993, that diltiazem does not illicit significant pharmacodynamic responses in healthy volunteers. Therapeutic equivalence of such formulations should therefore be established by pharmacodynamic evaluation or clinical studies using patients, and not in healthy subjects.

Lefebvre et al (1994) reported that no increase in SBP occurred after administration of SR formulation of diltiazem 120 mg. DBP and PR-intervals were decreased and increased respectively after administration of diltiazem SR. The results were not clinically significant. But in our study an increase as well decrease in all these PD parameters at different time points was observed. This may be attributed to the
absence of clinically significant effects in healthy volunteers or presence of active metabolites.

There is one report in literature (Paschoa et al, 1995) which utilizes prolongation of the PQ interval as a measure of therapeutic inequivalence between two formulations of diltiazem. The prolongation of the PQ interval (%age change) was taken as pharmacodynamic response. The 90% CI and T/R ratios were calculated and on this basis it was concluded that the two preparations of diltiazem were not bioequivalent. We in our study have also reached on the same conclusion based on PD parameters like SBP, DBP, sinus rate and PR-intervals.

In another study (Luckow & Paschoa, 1997), prolongation of the PQ interval as a measure of therapeutic inequivalence between two SR formulations of 180 mg diltiazem was used. The prolongation of the PQ interval (%age change) was taken as pharmacodynamic response. The 90% CI and T/R ratios were calculated and on this basis it was concluded that the two preparations of diltiazem were not bioequivalent. It was concluded that bioequivalence assessment of diltiazem is possible using pharmacodynamic parameters, however since, PK/PD relationships are influenced by the absorption rate, extent parameters may be misinterpreted when rate parameters of the test formulations are different. In our study also, based on PD parameters like SBP, DBP, sinus rate and PR-intervals, we have concluded that the formulations are not bioequivalent.

In another study reported in literature (Eradiri & Midha, 1997), it was concluded that the relationship between plasma diltiazem levels and pharmacodynamics are better investigated in controlled clinical trials using hypertensive patients.

A study has been reported in literature comparing the resting and effect of exercise on the PD of diltiazem (Yeung et al, 1999). In this study, SBP, DBP and HR were measured before, during and immediately after standard treadmill exercise according to Bruce protocol. The same measurements were done at steady state. It was concluded that diltiazem significantly decreased resting HR but not BP in healthy volunteers. It decreased both hemodynamic variables during exercise. Thus, the hemodynamic effects of diltiazem are more profound during exercise, and may be
more useful surrogate markers for calcium antagonists and other cardiovascular agents in healthy volunteer's studies. In our study also, we found that diltiazem significantly decreased resting HR but not SBP and DBP in healthy volunteers.

Overall, the results obtained in this study indicate that various formulations of diltiazem (narrow therapeutic index) marketed by different pharmaceutical companies in India may differ significantly in their bioavailability. The physicians prescribing these drugs, unsuspectingly presume that the quality of different formulations if not exactly the same, is nearly the same. The resultant sub-therapeutic response in many patients may be in fact due to significantly poor bioavailability from the particular formulation prescribed. Since most of practicing physicians in India do not have access to therapeutic drug monitoring, it is the legal as well as the moral duty of the manufacturers of the marketed formulation to ensure bioequivalence with the innovator's formulation, not only at the time of obtaining Regulatory Marketing Approval but also of subsequent batches of the formulations marketed by them over the years.

Regulations for Quality Assurance have been notified (Schedule Y) by the DCGI. However, the enforcement of these regulations in the Small Scale Sector Pharmaceutical companies (with over 20,000 establishment's), the largest number in the world has not been strictly carried out (Health being a State subject) Most of these units have no facility, whatsoever, to follow the Code of Good Manufacturing Practices, and hence it is no wonder that many of the formulations manufactured and marketed by these small manufacturers are found to be bioinequivalent to the innovator's formulations.
**Limitations of the study**

A large Intra-subject variability observed in this study indicates that a large sample size ought to have been used. Constraints of time and research funds in a thesis project precluded such a priori approach.

**Suggestions for future studies**

1. A higher dose of diltiazem should be used to elicit clinically significant pharmacodynamic responses.
2. Pharmacodynamic studies should be done in patients rather than in healthy volunteers especially in case of drugs like diltiazem.
3. A large sample size should be used to compensate for high intra-subject variability.
SUMMARY AND CONCLUSIONS

Medical profession has realized the problem of wide variations in the therapeutic effectiveness of various brands of oral formulations containing the same active ingredient in equal amounts.

Two products are generally considered to be bioequivalent if they yield comparable bioavailability when administered to the same individuals under similar conditions. The formulations deemed to be bioequivalent are therapeutically interchangeable.

In the present study, the pharmacokinetic and pharmacodynamic bioequivalence of three brands of diltiazem extended release capsules after single and multiple dose in healthy, male, adult, human subjects under fasting conditions was evaluated. The effect of drug administration on various Pharmacodynamic parameters after treadmill exercise was also studied.

The clinical study was carried out in accordance with ICH Good Clinical Practices. The study protocol and the informed consent form were approved by the Jamia Hamdard Institutional Review Board. Each of the subjects was required to understand and give his consent to participate in the study by signing the informed consent form. The signed original copy was retained. An adequate wash out period was maintained between the two treatment periods.

Bioequivalence was assessed by measuring the pharmacokinetic parameters namely $C_{\text{max}}$, $T_{\text{max}}$, $\text{AUC}_{0-4}$ and $\text{AUC}_{0-\infty}$ for diltiazem. Pharmacodynamic parameter namely $\text{AUC}_{0-4}$ for diltiazem.

Blood samples were collected within 2 minutes of the specified time as per study design. Intravenous indwelling cannula was kept in situ as long as possible, otherwise an alternative method of collecting by fresh clean veni punctures using standard disposable sterilized syringe and a needle was used. After collection, blood samples were centrifuged as per the processing method to separate plasma. All plasma samples were stored in suitably labeled polypropylene tubes at -70°C till analysis.
Blood pressure both Systolic and Diastolic (Korotkoff phase 5) was made at times specified in the protocol with the help of a mercury sphygmomanometer. A validated method in terms of selectivity, linearity, sensitivity, accuracy and precision was used for the estimation of diltiazem in plasma. The standard curve was linear & coefficient of co-relation was found to be greater than 0.99 throughout the study.

The bioequivalence criteria used were ratios of LSM of log transformed data for C\text{max}, T\text{max} and AUC\text{0-4} and the 90% confidence intervals, as per the DCGI guidelines.

Following conclusions can be drawn from the study:

1. Both the test products were not bioequivalent after single dose as well as after multiple dosing. Hence neither test product A nor test product B, could be substituted for reference product.

2. Product A showed greater variability at steady state while product B was less variable and was quite comparable to the reference (R).

3. Product A exhibited dose dumping both on Day1 and Day7. This could lead to toxic effects and to failure to maintain effective therapeutic concentration over 24 hr period in patients.

4. Product R fulfilled all the characteristics of ideal SR preparations Peak-Trough ratio >50%; takes maximum time to reach to 50% of C\text{max}; least variability in SS concentration; maximum C\text{min} concentration at SS; avoidance of undesirable high peaks of blood levels)

5. Though product B fared better than product A in some BE parameters. However, both the products were not bioequivalent to product R and hence cannot be substituted for product R, with confidence.

6. The order of quality of the products was as follows- R>B>A.
7. Only reference product exhibited P/T ratio > 50% at 24 hrs after administration of the last dose. None of the products tested met this goal 36 hrs after administration of the last dose. This has therapeutic implications if a patient on diltiazem SR happens to miss a dose. Therefore, serious attention must be paid to ensure patient's compliance to treatment especially when SR formulations are prescribed.

8. The 90% confidence intervals as well as T/R ratios of none of the formulations (Day1 & Day7) were within the prescribed limit of bioequivalence for any of the pharmacodynamic parameters i.e. PR-interval, QTc-interval, HR, SBP or DBP. Hence none of the test products were pharmacodynamically bioequivalent to the reference product. Overall, diltiazem increased exercise tolerance and significantly decreased HR but not SBP and DBP in healthy volunteers after treadmill exercise test. The statistically significant differences observed with all the 3 products were not large enough to be of clinical significance.

9. Normal human subjects are not suitable for testing pharmacodynamic bioequivalence of diltiazem formulations because of ethical constraints. These studies must, therefore, be carried out in patients.


Christ, P. V. (1985) Inhibition of hepatic microsomal drug metabolism by the calcium channel blockers diltiazem and verapamil. Biochem. Pharmacol. 34: 2549-2555


172


Nomier, A. A. (1999) Improved reliability of the rapid microtiter plate assay using recombinant enzyme in predicting CYP2D6 inhibition in human liver microsomes. Drug Metab. Dispos. 27: 436-439


