

A Comparative Single Dose Bioequivalence Study of Extended Release Antihypertensive Drug Formulation among Healthy Human Volunteers



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ABSTRACT

The objective of this study was to compare the in vivo characteristics of diltiazem extended release formulations for once daily, which were expected to be bioequivalent. Either two capsules of a test formulation or a 1 of the reference formulation, both containing 360 mg diltiazem were administered to healthy male volunteers after keeping fast of ten hour in a randomized, open label, three period crossover design. Plasma samples obtained over the subsequent period of 72 hours were analyzed using a validated LC-MS/MS method. Safety profile and tolerability of the study medications were assessed by analysis of adverse events obtained by vital sign measurements, electrocardiography, and clinical. The 90% CI for the log transformed data for Cmax, AUC0-t, AUC0-∞ for both the test product fell in the prescribed limits of bioequivalence for narrow therapeutics index drugs i.e. 80 to 120%. This single dose study found that the test and reference products met the regulatory criteria for bioequivalence in healthy, male volunteers under fasting.

Key words: Diltiazem, bioequivalence, bioavailability

Sağlıklı Gönüllü İnsanlar Arasında Uzun Salınlı Antihipertansif İlaç Formülasyonunun Bir Karşılaştırmalı Tek Doz Biyoşdeğerlik Çalışması

ÖZET

Bu çalışmanın amacı, biyolojik olarak eşdeğer olması beklenen, günde bir defalık diltiazem uzatılmış salınlı formülasyonlarının in vivo özellikleri karşılaştırmaktır. Her ikisi de 360 mg Diltiazem ihtiva eden bir test formülasyonun iki kapsülü veya referans formülasyonun 1 kapsülü, on saat aç bekletildikten sonra sağlıklı erkek gönüllülere randomize, açık etiketli, üç periyotlu çapraz tasarımda verilmiştir. Üzerinden 72 saat süre geçtikten sonra elde edilen plazma numuneleri geçerli bir LC-MS/MS yöntemi kullanılarak analiz edildi. Çalışma ilaçlarının güvenlik profili ve tolere edilebilirliği vital bulguların ölçümü, elektrokardiyografi ve klinik ile elde edilen yan etkilerin analizi ile değerlendirildi. Cmax log dönüştürülmüş verileri için 90% CI, AUC0-t, AUC0-∞ her iki ilaç için dar terapötik indeks ilaçlar için biyoşdeğerliliğin öngörülen limitleri içine düştü, örneğin 80- 120%. Bu tek doz çalışma tespit etmiştir ki test ve referans ürünler açlık altında sağlıklı, erkek gönüllülerde biyoşdeğerlik için düzenleyici kriterleri karşılamaktadır.

Anahtar Kelimeler: Diltiazem, biyoşdeğerlik, biyoyararlanım

INTRODUCTION

Drug administered orally or parenterally must reach the general circulation in their pharmacological active form to be distributed throughout the body and to exert therapeutic effect. The intensity of therapeutic actions of many drugs correlate well with the concentration of the drug in the biological fluid (1). The rate of absorption is therapeutically important in case of narrow therapeutic index drugs, (2) where relatively small changes in the concentration can lead to marked changes in action of drug.

Diltiazem is a narrow therapeutic index drug and exhibits dose-dependent pharmacokinetics. Diltiazem is a potent vasodilator but does not usually cause reflex tachycardia. It reduces coronary as well as peripheral vascular resistance, (3,4) causing a decrease in blood pressure, and also decreases heart rate and myocardial oxygen demand. In addition, it is extremely well tolerated by patients. These characteristics make diltiazem well suited for the treatment of systemic hypertension. Although the effectiveness of diltiazem for the treatment of patients with hypertension has been well demonstrated in numerous placebo-controlled (5-9) and comparative (10-19) clinical trials, most physicians have had some concern about its efficacy and have used it predominantly in patients with mild hypertension.

Clinical studies compare the diltiazem hydrochloride extended release capsules (Wockhardt Pvt.Ltd.) with the CARDIZEM LA Capsule (Biovail Laboratories, USA). The forms of the drug were shown to have similar trends in half life despite the difference in absorption rate. It was found that 95% of the extended release capsule is absorbed throughout the dosing interval. The capsules

takes effect within two to three hour and active effect are detected for 10 to 14 hour.

CARDIZEM® LA (diltiazem hydrochloride) is a calcium ion cellular influx inhibitor (slow channel blocker or calcium antagonist). Chemically, diltiazem hydrochloride is 1,5-benzothiazepin-4 (5H)-one, 3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride, (+)-cis-.

Diltiazem hydrochloride is a white to off-white crystalline powder with a bitter taste. It is soluble in water, methanol and chloroform. It has a molecular weight of 450.99. It undergoes extensive presystemic metabolism (20), and the absolute bioavailability is approximately 40%, showing large interindividual variation. Diltiazem is a drug with a short half-life, so rapid release diltiazem preparations are required to be administered in multiple daily doses, which may lead to poor patient compliance and hence inadequate therapeutic response. In order to overcome these problems, extended release (SR) preparations of diltiazem have been developed and marketed.

Most of the bioequivalence studies on which the claims of bioequivalence to innovator product do not use confidence intervals (CI). Determination of CI is a current regulatory requirement of DCGI (Drug Controller General of India) and also of FDA' (<http://www.fda.gov/gov/cder/guidance/index.htm>; April, 2003) to document bioequivalence. Thus, the only way to verify these claims is to do a comparative bioequivalence study with the innovator drug formulation, using confidence intervals.

Hence, the present study was undertaken to compare the bioavailability of three brands of 360 mg extended release diltiazem in healthy, adult, male, human subjects under fast-ing conditions.

MATERIAL AND METHODS

In Vitro Dissolution

In vitro dissolution characteristics of the study drugs were determined prior to the clinical study to determine a possible lack of robustness of the formulations. Therefore, tablets of each formulation were dissolved in four different buffer media (0.1 M hydrochloric acid, pH 1; acetate buffer, pH 4.5; phosphate buffer, pH 6.8; and phosphate buffer, pH 8) covering the entire pH range of the gastrointestinal tract under the addition of 1% sodium dodecyl sulfate to achieve sink conditions.

Table 1. Randomization

Subject No.	Sequence	Time (hrs)	Period		
			Period -1	Period-2	Period-3
01	CAB	0900	C	A	B
02	BCA	0902	B	C	A
03	ABC	0904	A	B	C
04	ABC	0906	A	B	C
05	CAB	0908	C	A	B
06	BCA	0910	B	C	A
07	ABC	0912	A	B	C
08	BCA	0914	B	C	A
09	CAB	0916	C	A	B
10	CAB	0918	C	A	B
11	BCA	0920	B	C	A
12	ABC	0922	A	B	C
13	CAB	0924	C	A	B
14	ABC	0926	A	B	C
15	BCA	0928	B	C	A
16	CAB	0930	C	A	B
17	BCA	0932	B	C	A
18	ABC	0934	A	B	C

A=Test drug product. B=Test drug product. C= Reference drug product

Investigations were performed in a standard paddle apparatus 24 with a rotation speed of 100 rpm in vessels of 900 mL over the time range of 24 hours.

Clinical Study

The design of the study was open-label, randomized, and controlled and followed a three period crossover with single oral doses of either one 360-mg capsules of the test formulation (A&B) or one 360-mg capsule of the reference formulation(C)table1, with a treatment-three phase of at least seven days to avoid any carryover effects in the second period. This exploratory trial was performed in 18 healthy volunteers without a formal sample size estimation as the number was considered sufficient to fulfill the objectives of the study. The investigation was performed in healthy males only as there have been no reports of gender-specific differences in diltiazem pharmacokinetics. Subjects were included according to specific inclusion and exclusion criteria, taking into account both participants' safety and optimal standardization of the study. Subjects with any clinically relevant laboratory parameters out of range; clinically relevant findings in ECG or vital signs; existing cardiac, hematologic, hepatic, renal, gastrointestinal diseases or findings; clinically relevant diseases of the internal organs or central nervous system; severe allergies or hypersensitivities or who had undergone a

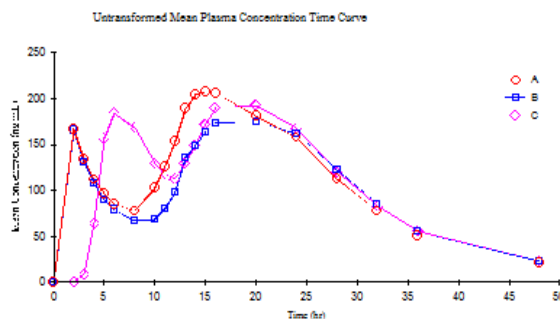


Figure 1. Plasma diltiazem concentration versus time of brand.

clinically relevant blood donation or participation in a clinical trial during the last months prior to the start of the study were excluded. Any medical disorder, condition, or history of such that would impair the subject's ability to participate or complete this study with a special focus on effect of absorption and metabolism led to exclusion of a subject. Furthermore, subjects were excluded if they had regular intake of alcohol ≥ 50 g pure ethanol per day or caffeine ≥ 250 mg/d, were active smokers, and/or had received any systemically available medication within four weeks prior to the intended first study drug administration unless, due to the corresponding terminal elimination $t_{1/2}$ values, complete elimination from the body for the drug and/or its primary metabolites could be assumed. Finally, drug or alcohol dependence and a positive virologic status (anti-HIV test, HBsAg test, or anti-HCV test) were to be excluded. Prior to the start of administration of the investigational products a pre-study examination was performed to determine the general health status of the subjects. It included an anamnesis for medical history, a physical examination, determination of blood pressure and pulse rate (oscillometry using a manual noninvasive device), a twelve lead ECG, determination of hematologic and clinical chemistry parameters, and a urinalysis (the latter performed by a Good Laboratory Practices- certified central laboratory using common and quality controlled standard methods for determination).

Hospitalizations started twelve hours before study drug administration in each period and lasted for 48 hours post dosing. Drug administration was performed under standardized conditions in an sitting position with 240

Table 2. Demographic Characteristics of the 18 Study Participants

	Age (y)	Body weight(kg)	Height(cm)
Mean±standard deviation	21.6±2.9	71±13.3	173±7.9
Minimum	18.0	53.0	162.0
Maximum	40.0	89.0	185.0

ml. tap water by maintaining an overnight fast (at least 10 hours). Standardized meals were served 4, 8, 12, 24, 28, and 32 hours post administration, which had the same standardized composition in three periods. Water restriction one hour pre-dose and two hours post-dose except the water given at the time of dosing had maintained. Volunteers had to remain in a sitting position for four hours after administration.

Venous blood samples were collected immediately before and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after drug administration. The collected blood samples were immediately chilled; the plasma was separated by centrifugation in a refrigerated centrifuge, and set at 4 °C with a rate of 3500 rpm for 10 minutes. The separated plasma was then immediately shock-frozen using liquid nitrogen and then stored at -70 °C until assay. The stability of these samples at -70 °C is three months (21).

Questioning for general well-being was performed in a non-leading manner. In addition to the questioning for general well-being at the pre-study examination and at the time of hospitalization, questioning for general well-being was also performed in the morning prior to the study drug administration as well as 1, 4, 8, 12, 24, 36, and 48 hours post administration and at the post-study examination. Blood pressure and pulse rate were measured in the morning prior to the study drug administration as well as 1, 2, 4, 8, 12, 24, 36, and 48 hours post administration. Furthermore, the volunteers were asked to report any adverse events spontaneously, whether or not they occurred during confinement. The entire trial was performed in accordance with the requirements of Good Clinical Practices and the current version of the Declaration of Helsinki (22,23)

Each volunteer provided written informed consent, which could be withdrawn at any time. The design and procedures corresponded to recommendations of international guidelines. The study was approved by the Ethics Committee of the Wockhardt Ltd, Mumbai, India.

Pharmacokinetic Parameters and Statistical Analysis

The pharmacokinetic characteristics of extended release diltiazem were determined from the plasma concentration-time data. Peak plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) were determined directly from raw data. The area under the curve (AUC_{0-t}, from 0 to last measured concentration) was calculated.

All the pharmacokinetic parameters statistical values were calculated using LinMax procedures of WinNolin® Version 5.1 (Pharsight Corporation USA) software application and the SAS® system Version 9.1, respectively, at Clinical Pharmacokinetic & Biopharmaceutics Department of Wockhardt Ltd and 95% confidence interval analysis with a minimum level for significant difference set at P < 0.05. All data were reported as mean standard deviation.

RESULTS

Study Population

A total of 18 volunteers were enrolled and finished the study according to the protocol without major protocol deviations. The median age was 28.0 years (range, 18-42 years), the mean weight was 70.2 kg (range, 58.5-80.0 kg), and the mean body mass index was 24.2 kg/m² (range, 19.3-27.0 kg/m²) (Table 2).

Pharmacokinetics and Statistics

The clinical study was completed within four weeks. Extended release diltiazem was well tolerated by subjects, and no adverse events occurred during the study. Mean pharmacokinetic parameters for the 18 subjects for the extended release Diltiazem tested formulations and the reference formulation are shown in Table 3 and 4. The time course of mean Diltiazem concentrations after 360 mg for both formulations is presented in Figure 1.

Pharmacokinetic Analysis

Peak Plasma Concentration (C_{max}) or Peak Exposure

Parent Drug C_{max}: The C_{max} for test products A ranged from Mean±SD of 443.09±62.389 ng.hr/ml and the AUC_{0-t} for test products B ranged from Mean±SD of 338.85±62.389 ng.hr/ml. The AUC_{0-t} for reference product C ranged from Mean ±SD of 307.95±50.038 ng.hr/ml. The geometric values for the test products A, test

products B and reference product C were found to be 211.29 ng.hr/mL & 193.33 ng.hr/mL and 220.58 ng.hr/mL, respectively.

Time of Peak Concentration (Tmax)

Parent Drug Tmax: The Tmax for test products A ranged from Mean \pm SD of 20.00 \pm 4.78 ng.hr/ml and the AUC_{0-t} for test products B ranged from Mean \pm SD of 28.00 \pm 8.513 ng.hr/ml The AUC_{0-t} for reference product C ranged from Mean \pm SD of 20 \pm 5.8 ng.hr/ml. The geometric values for the test products A, test products B and reference product C were found to be 15.00 ng.hr/mL and 18.00 ng.hr/mL and 15.50 ng.hr/mL, respectively.

Area Under the plasma Concentration time curve (AUC_{0-t}, t=72hr) and AUC_{0-∞}

Parent Drug AUC_{0-t}: The AUC_{0-t} for test products A ranged from Mean \pm SD of 8827.29 \pm 1635.529 ng.hr/ml and the AUC_{0-t} for test products B ranged from Mean \pm SD of 8303.1900 \pm 1370.553 ng.hr/ml The AUC_{0-t} for reference product C ranged from Mean \pm SD of 8176.60 \pm 3029.7850 ng.hr/ml. The geometric values for the test products A, test products B and reference product C were found to be 4844.60ng.hr/mL and 4558.71ng.hr/mL and 4838.91 ng.hr/mL. respectively.

Parent drug AUC_{0-∞}: The AUC_{0-∞} for test product A and test product B ranged from Mean \pm SD of 9427.0419 \pm 1805.079ng.hr/ml and 8832.9560 \pm 1494.748 ng.hr/ml respectively. The AUC_{0-∞} for reference product C ranged from a Mean \pm SD of 8894.3815 \pm 1660.006 ng.hr/ml. The geometric values for the test products A and test products B and reference products C were found to be 5082.86ng.hr/mL & 4813.06ng.hr/mL and 5101.52ng.hr/mL.

Elimination Rate Constant (K_{el})

Parent Drug: The Mean \pm SD values of the elimination rate constant (K_{el}) were found to be 0.1315 \pm 0.024 hr⁻¹ and 0.1232 \pm 0.016hr⁻¹ for Test Product A and test products B respectively and 0.1297 \pm 0.021hr⁻¹ for Reference Product C. The geometric mean values for both the test products A & test products B & Reference Products C were found to be 0.09 hrs⁻¹ and 0.09 hrs⁻¹ and 0.09hrs⁻¹ respectively.

Elimination Half-life (t_{1/2})

Parent Drug: The Mean \pm SD values of elimination half-life (t_{1/2}) were found to be 13.0008 \pm 2.487hrs&11.1

026 \pm 1.500for Test Product A and test products B and 12.2680 \pm 2.010hrs for Reference Product C.

Parent Drug: The Median half-life (t_{1/2}) values for the Test A and test products B and Reference Products C were found to be 7.66hrs and 8.01 hrs and 8.07 hrs, respectively.

Residual Area (AUC_%Extrap_obs)

Parent Drug: The Mean \pm SD values of the Residual Area (%) were found to be 12.40 \pm 3.795& 10.86 \pm 2.458 for Test Product A & test products B and 12.01 \pm 3.174 for Reference Product C.

Statistical Results

Geometric LSM Ratio and 90 % Confidence Interval

The test by reference geometric least square mean ratio and 90 % confidence interval obtained for C_{max}, AUC_{0-t} and AUC_{0-∞} were as follows:

Parent drug (A): LSM ratio C_{max} 95.79% and CI 88.52% to 103.65%, AUC_{0-t} LSM ratio 92.85% and CI 92.85% to 107.96% and AUC_{0-∞} ,LSM ratio 99.63% and CI 92.2% to 107.67%, which shows all the values are within the bioequivalence acceptance range 80.00%'to 125.00% .

Parent drug (B): LSM ratio C_{max} 87.65% and CI 81% to 94.84%, AUC_{0-t} LSM ratio 94.21% and CI 87.37% to 101.59% and AUC_{0-∞} LSM ratio 94.35% and CI 87.31% to 101.95%, which shows all the values are within the bioequivalence acceptance range 80.00%' to 125.00%

p-values (ANOVA)

The p-value should be greater than 0.05 for C_{max}, AUC_{0-t} and AUC_{0-∞} for period and formulation effects. For sequence effect it should be greater than 0.01.

The p-values obtained from ANOVA for sequence effect of Drug Diltiazem hydrochloride and metabolite of Drug Diltiazem hydrochloride are greater than 0.05 for C_{max} , AUC_{0-t} and AUC_{0-∞} which indicates no statistically significant differences were observed for sequence effect on pharmacokinetic parameters C_{max} , AUC_{0-t} and AUC_{0-∞}.

Intra-subject Variability

Parent Drug (A): The coefficients of variation (CV%) corresponding to intra-subject variability for C_{max}, AUC_{0-t} and AUC_{0-∞} for Drug Diltiazem hydrochloride are 14.03%,13.41%,13.79% respectively which were found to be less than 30%.

Parent Drug (B): The coefficients of variation (CV%) corresponding to intra-subject variability for Cmax, AUC_{0-t} and AUC_{0-∞} for Diltiazem hydrochloride are 14.03%, 13.41%, 13.79% respectively, which were found to be less than 30%.

Power

Parent Drug (A): The power values obtained for Cmax, AUC_{0-t} and AUC_{0-∞} are 98.84%, 99.86%, 99.74% respectively, which were greater than 80.00% the desired power to support the bioequivalence test, and hence test, and hence considered to be adequate for supporting bioequivalence conclusions.

Parent Drug (B): The power values obtained for Cmax, AUC_{0-t} and AUC_{0-∞} are 98.84%, 99.86%, 99.74% respectively, which were greater than 80.00 % the desired power to support the bioequivalence test, and hence test, and hence considered to be adequate for supporting bioequivalence conclusions

Safety Results

There was one adverse events reported which was mild fever Subject no. 11 adverse events was resolved. The adverse event was mild and unlikely to study medication administered to the subjects. From the adverse event profile and tolerability of the subjects, it appeared that the test product was equally safe as that of reference product.

DISCUSSION

Proving two drug products (of the same active ingredient) to be therapeutically equivalent entails a similarity in rate and extent to which a drug in a dosage form becomes available for biologic absorption (24). Area-under-the-curve is accepted as a good indicator of extent of absorption, whereas Cmax and Tmax are considered estimators of the rate of absorption. Two internationally recognized organizations (U.S. Food and Drug Administration and European Agency for the Evaluation of Medicinal Products) have proposed that bioequivalence can only be assumed when the characteristic parameters of bioavailability show no more than a defined difference (25-26). These differences depend on the nature of the drug, the patient population, and the clinical end point.

The rapid hydrolysis was minimized by working under

low temperature at all times using liquid nitrogen to shock-freeze the samples and stop hydrolysis, thawing in chilled ice water, and centrifuging in a refrigerated centrifuge set at 4°C. Such methods helped us bypass the need to add an enzyme inhibitors (such as potassium fluoride or physostigmine) to plasma to enzymatically inhibit hydrolysis. According to earlier investigations such preservatives are not very efficient, because enzymatic hydrolysis in plasma overlaps with chemical hydrolysis. Therefore, immediate cooling techniques used in this study, including storage at -70°C after sampling, are the best steps to prevent degradation. Lack of statistical significant differences in AUC values, Cmax and, Tmax between the two products indicate that the two formulations are closely similar in terms of their pharmacokinetic properties and bioavailability. This suggests that the in vivo dissolution and the absorption rate are closely identical for the two products. Furthermore, this in vivo finding is consistent with the in vitro release pattern.

Based on the in vitro and in vivo pharmacokinetic results obtained, this study suggests that the two products of extended release diltiazem included in this investigation are bioequivalent. Thus, diltiazem hydrochloride and CARDIZEM LA might be considered interchangeable based on the pharmacokinetic effect.

REFERENCES

1. Koch-Wesser J. Serum drug concentrations as therapeutic guides. *N Engl J Med* 1982;287:227-31.
2. Benet LZ, Goyan JE. Bioequivalence and narrow therapeutic index drugs *Pharmacotherapy* 1995;15:433-40.
3. Buckley MM-T, Grant SM, Goa KL, et al. Diltiazem: A reappraisal of its pharmacologic properties and therapeutic use. *Drugs* 1990;39:757-806.
4. Katzung BG, Chatterjee K. Vasodilators and the treatment of angina pectoris. In: Katzung BG, ed. *Basic Clinical Pharmacology*. 4th ed. Norwalk, Conn: Appleton & Lange; 1989:140-51.
5. Nikkila MT, Inkovaara JA, Heikkinen JT, Olsson S-ORI. Antihypertensive effect of diltiazem in a slow-release formulation for mild to moderate essential hypertension. *Am J Cardiol* 1989;63:1227-30.
6. Andrtin L, Htglund P, DotevaU A, et al. Diltiazem in hypertensive patients with type II diabetes mellitus. *Am J Cardiol* 1988;62:114G-20G.
7. Ginsberg D, Pappas JE, Rofman BA, et al. Once-daily dosing of sustained-release diltiazem capsules in mild-to-moderate hypertension. *J Am Osteopath Assoc* 1988;88:1489-92.

8. Pool PE, Massie BM, Venkataraman K, et al. Diltiazem as monotherapy for systemic hypertension: A multicenter, randomized, placebo-controlled trial. *Am J Cardiol* 1986;57:212-7.
9. Pool PE, Herron JM, Rosenblatt S, et al. Metabolic effects of antihypertensive therapy with a calcium antagonist. *Am J Cardiol* 1988;62:109G-13G.
10. Szlachcic J, Hirsch T, Tubau JF, et al. Diltiazem versus propranolol in essential hypertension: Responses of rest and exercise blood pressure and effects on exercise capacity. *Am J Cardiol* 1987;59:393-9.
11. Frishman WH, Zawada ET Jr, Smith LK, et al. Comparison of hydrochlorothiazide and sustained-release diltiazem for mild to moderate systemic hypertension. *Am J Cardiol* 1987;59:615-23.
12. Leary WP, van der Byl K. Diltiazem compared with hydrochlorothiazide in the treatment of mild-to-moderate essential hypertension. *S Afr Med J* 1988;74:13-5.
13. Leehey WE, Hartman E. Comparison of diltiazem and hydrochlorothiazide for treatment of patients 60 years of age and older with systemic hypertension. *Am J Cardiol* 1988;62:1218-3.
14. Frishman WH, Charlap S, Goldberger J, et al. Comparison of diltiazem and nifedipine for both angina pectoris and systemic hypertension. *Am J Cardiol* 1985; 56:41H-6H.
15. Schulte K-L, Meyer-Sabellek WA, Haetenberger A, et al. Antihypertensive and metabolic effects of diltiazem and nifedipine. *Hypertension* 1986;8:859-65.
16. Simic D, Jurman V. Effects of diltiazem and nifedipine on blood pressure. *J Cardiovasc Pharmacol* 1988;12(Suppl):S220-1.
17. Török E, Wagner M, Podmaniczky M. Comparison of urapidil and atenolol in hypertension. *Drugs*. 1988;35 Suppl 6:164-72.
18. Massie B, MacCarthy EP, Ramanathan KB, et al. Diltiazem and propranolol in mild to moderate essential hypertension as monotherapy or with hydrochlorothiazide. *Ann Intern Med* 1987;107:150-7.
19. Weiss RJ, Bent B. Diltiazem-induced left ventricular mass regression in hypertensive patients. *J Clin Hypertens* 1987;3: 135-43.
20. Buckley MM-T, Grant SM, Goa KL, McTabish D, Sorkin EM. Diltiazem. A reappraisal of its pharmacological properties and therapeutic use. *Drugs* 1990;39:757-806.
21. CDSCO (Central Drug Standard Control Organization), 2005, Guidelines for Bioavailability and Bioequivalence. Retrieved from <http://cdsco.nic.in/html/BE%20Guidelines%20Draft%20Ver10%20March%2016,%202005.pdf>, accessed on 12 March 2008
22. European Medicines Agency, ICH Topic E6 (R1) Note for guidance on good clinical practice (CPMP/ICH/135/95), 2002.
23. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; Note of Clarification on Paragraph 29, Washington 2002; Note of Clarification on Paragraph 30, Tokyo 2004.
24. Chow CS, Liu JP: Design and analysis of bioavailability and bioequivalence studies. New York: Marcel Dekker; 1992:1-2.
25. US Food and Drug Administration (FDA): Guidance: statistical procedure for bioequivalence studies using standard 2-treatment cross-over design. Statement under 21 CFR 10.9. Rockville, MD: US Department of Health and Human Services; 1992.
26. Committee for Proprietary Medicinal Products (CPMP) Working Party on the Efficacy of Medicinal Products: Note for guidance: investigation of bioavailability and bioequivalence. Brussels; 1991.