Research Article

Determination of Simvastatin and Diltiazem in Rat's Plasma by HPLC and Pharmacokinetic Studies

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Accepted on: 03-04-2013; **Finalized on:** 30-06-2013.

ABSTRACT

To develop a simple and sensitive high performance liquid chromatographic method for the simultaneous estimation of Simvastatin (SIMVA) and Diltiazem (DTZ) in rat plasma and also to calculate the possible pharmacokinetic parameters. The standard cholesterol diet was used to induce hyperlipidemia in Wistar rats (300±40 gm). The blood samples were collected (on 1st and 8th day) from SIMVA (2 mg/kg) alone and in combination with DTZ (15 mg/kg) treated groups and were analyzed for various pharmacokinetic parameters. A good linearity was found to be in the range of 0.1-50µg/mL for the two drugs Simvastatin and Diltiazem, with r^2 value of 0.995. The accuracies for intra-day and inter-day precisions were ranged from 96.6% to 98.4% over the concentration range of 0.1 to 50µg/mL of two drugs. This developed method was rapid, sensitive, reproducible and successfully applied for studying the pharmacokinetic interaction between these two drugs.

Keywords: Diltiazem, Hyperlipidemia, Pharmacokinetic parameters, Simvastatin.

INTRODUCTION

Simvastatin [(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-hydroxy 6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl2,2-dimethylbutanoate] belongs to the statin drug family, the members of which are used as cholesterol lowering agents for patients with hypercholesterolemia.[1] It is used in the treatment of hyperlipidemia or cardiovascular complications like coronary heart disease along with calcium channel blocker (CCB).

Diltiazem[cis-(+)-[2-(2-dimethylaminoethyl)-5-(4-methoxy phenyl)-3-oxo-6-thia-2 azabicyclo [5.4.0] undeca-7, 9, 11-trien-4-yl] ethanoate] is a calcium channel blocker (CCB) widely used in the treatment of angina and hypertension, may have potential stroke preventing effect and used in ischaemic heart disease.[2,3] It is extensively metabolized in humans by CYP450s yielding a host of metabolites some of which have potent pharmacological activities which may contribute to the antihypertensive and antiischemic properties of the parent DTZ in clinical therapy.[4, 7]

MATERIALS AND METHODS

Simvastatin (Figure 1) and Diltiazem (Figure 2) are obtained as gift samples from Aurabindo Labs, Mahabubnagar, AP, India. HPLC grade water, acetonitrile, orthophosphoric acid were obtained from Finar chemicals Ltd Ahmedabad, diethyl ether, potassium hydroxide, perchloric acid 60% were purchased from Qualingers fine chemicals, Mumbai, India.

Chromatographic systems

Shimadzu HPLC (Model LC-20AT contain C18 column coated with 5 micron particles), Colorimeter (Remi), Centrifuge (Remi), Refrigerator (Blue star) Vortex mixer (Nelox), Sonicator (Hwashin technology, Korea).

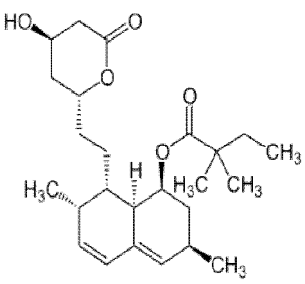

Figure 1: Simvastatin

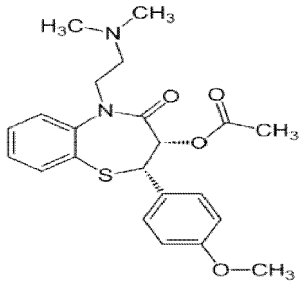

Figure 2: Diltiazem

Chromatographic Conditions

Mobile phase consists of Acetonitrile:Water:Ortho phosphoric acid at 65:35:0.1% v/v (adjusted to pH 2.8 with Ortho phosphoric acid), Flow rate - 1mL/min, Volume of injection - 20µL, Retention Time (minutes)- Simvastatin - 3.88min, Carbamazepine (internal standard) - 5.91mins. Before using the mobile phase, it was degassed by passing it through a 0.45µm filter. The mobile phase was pumped at an isocratic flow rate of 1.0 mL/min at room temperature. The UV detection wavelength was set at 235nm and sensitivity of 0.001 AUFS was used for the analysis.

Extraction procedure

Rat plasma samples were prepared for chromatography by precipitating proteins with 0.1mL perchloric acid (60%) for each 0.5mL of plasma. The resultant solution was mixed for 5min on vortex shaker at room temperature and centrifuged at 3000-5000 rpm for 10 min. The Supernatant was separated. To 300µL of the supernatant 2µL internal standard was added. Then add 5mL diethyl ether and 1mL KOH (4M). This solution was vortexed for 5min, centrifuged at 3000-5000 rpm for 10min., collected the supernatant and evaporated it to 4-6 hrs under nitrogen stream. The residue was collected and 100µL of

mobile phase was added to it. From this 20 µL of the solution was used for HPLC Analysis.

Calibration curves

Aliquots of 0.5 mL of blank plasma were spiked with 50 µL working standard solutions of two drugs yielding final concentrations of 0.1, 10, 20, 30, 40 and 50µg/mL. To 50µL of these solutions, 450µL of blank plasma and 10µL of internal standard (IS) carbamazepine (2 µg/mL) were added and vortexed for 5 minutes. Study sample was treated with 2.5mL of ice-cold absolute ethanol for each 0.5 mL of plasma for protein precipitate and remaining extraction procedure was followed as above. The peak areas of Simvastatin, Diltiazem and Carbamazepine were calculated. The peak area ratios obtained for different concentrations of the Simvastatin and Diltiazem were plotted against the concentration of two drugs. The slope of the plots determined by the method of least square regression analysis (r^2=0.995) was used to calculate the Simvastatin and Diltiazem concentration in the unknown sample.

Accuracy and Precision

Intraday reproducibility was tested by using different concentrations (0.1, 1, 10µg/mL). The procedure was repeated on three separate days to allow determination of inter-day precision and accuracy. Intraday accuracy was estimated based on the mean % error and inter-day accuracy was calculated as the mean of the intraday accuracy determinations. The precision expressed as a%, was evaluated by calculating the intra and inter day relative standard deviations.

Extraction recovery

The extraction efficiency was determined by comparing the peak area ratios of known amounts of two drugs (un extracted) in mobile phase to that of samples containing the same amounts of two drugs in plasma after extraction.

Study design

Adult male wistar albino rats weighing 180-200g were selected and allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. The standard cholesterol diet along with butter (0.5 mL twice a day) was administered for 30 days to induce hyperlipidemia.[8] At the end of one month the blood was withdrawn by retro orbital puncture to analyze lipid profiles (TC, TG, LDL-C and HDL-C) to confirm the induction of hyperlipidemia. The hyperlipidemic rats were divided into three groups of six rats in each group (n=6).

Group I: (HL) Control group of Hyperlipidemic rats received a dose of 1.5% CMC.

Group II: (SIMVA) simvastatin (2 mg/kg) alone in Hyper lipidemic (HL) rats.

Group III: (DTZ) Diltiazem (15 mg/kg) alone in HL rats.

Group IV: (SIMVA-DTZ) Simvastatin (2 mg/kg) in combination with Diltiazem (15 mg/kg).

This study was carried out for 8 days and the protocol of the present study was approved by the Institutional Animal Ethical Committee (IAEC No: 2012/11/1/S-I/17), Vaagdevi College of Pharmacy, Hanamkonda, AP. India.

Analysis of study samples

Study samples were obtained from rats, which were treated with Simvastatin and Diltiazem since one week. Six blood samples were collected at 0.5, 1, 2, 4, 8 and 24 h post oral dose of drug administration. Plasma was separated and stored at -40°C until analysis.

Statistical analysis

Pharmacokinetic parameters like peak plasma concentration (C_{max}), time to reach peak concentration (T_{max}), area under the curve [AUC], elimination half life ($t_{1/2}$), mean retention time [MRT], total clearance [CL/f] and volume of distribution [V_d] were calculated for each sample using a non compartmental pharmacokinetic model. The plasma levels of Simvastatin in SIMVA and SIMVA-DTZ groups at different time points, on day 1 and day 8.

RESULTS AND DISCUSSION

Typical chromatograms for simultaneous estimation of Simvastatin and Diltiazem in rat plasma were shown in Figure 3. The retention times of Simvastatin, Diltiazem and Carbamazepine (internal standard) were approximately 3.91, 9.36, and 5.51 minutes respectively. The analytical run time was 15 min for each plasma sample. The mean extraction efficiencies of Simvastatin and Diltiazem from serum at a concentration of 0.1-50µg/mL were 90-96%.

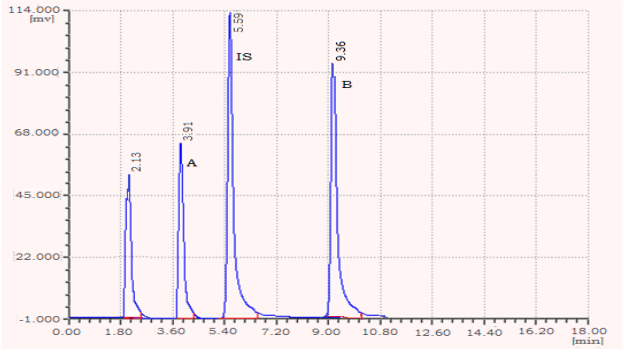

Figure 3: Typical HPLC chromatogram for the simultaneous estimation of Simvastatin and Diltiazem in rat plasma. A= Simvastatin; B= Diltiazem; IS= Internal Standard (Carbamazepine).

Method validation

The regression equation for Simvastatin and Diltiazem were Y = 0.053 X - 0.029 and Y= 0.033 X + 0.003 (Y= peak area ratio and X = concentration) respectively. The linearity was found to be in the range of 0.1-50µg/mL for two drugs, with r^2 value of 0.995. The calibration curve passes through the origin, which justifies the use of single

point calibration. Over the range of concentrations from 0.1-50µg/mL, the intraday accuracies ranged from 97.00% to 98.68% and 96% to 97.98% for SIMVA and DTZ respectively. The average inter-day accuracies were ranged from 95.00% to 98.02% and 95.60% to 97.88% for SIMVA and DTZ respectively. The HPLC method described here was accurate and precise and capable of determining concentrations of Simvastatin and Diltiazem in small volumes of rat serum. The extraction procedure was simple and procedure used an easily available internal standard carbamazepine.

Pharmacokinetics

The mean plasma concentration time curves after oral administration of Simvastatin and Diltiazem drugs in SIMVA and DTZ groups (on day 8) were shown in Figure 4 & 5 and corresponding pharmacokinetic parameters were shown in Table 1 and 2. There is a significant difference in the pharmacokinetic parameters of Simvastatin in presence of Diltiazem and vice versa on day 8 in hyperlipidemic rats. These findings suggest that pharmacokinetic interaction between Simvastatin and diltiazem in hyperlipidemic rats.

Table 1: Pharmacokinetic parameters of simvastatin alone and in presence of Diltiazem in hyperlipidemic rats on day 8 (Mean ± SD, n=6)

Parameters	Simvastatin alone on day 1	Simvastatin alone on day 8	Significance
C_{max}(µg/mL)	13.02 ± 0.46	14.36 ±0.32	NS
T_{max}(Hrs)	2.00 ± 0.00	2.00 ± 0.00***	YES
AUC(tot)	50.12 ± 1.37	53.43 ± 2.04	NS
$T_{1/2}$ (Hrs)		2.00 ± 0.17	NS
MRT(min)	3.77 ± 0.25	3.68 ± 0.12	NS
CL/f(mL/kg)	160.56 ± 4.52	151.62 ± 4.92	NS
V_d (mL)	473.59 ± 37.76	438.51 ± 18.87***	YES

*Significant at P<0.05; ** Significant at P<0.01; ***significant at P<0.001 compared to Simvastatin alone day 1 (One way ANOVA followed by Bonferroni's test).

Table 2: Pharmacokinetic parameters of Diltiazem alone and in presence of Simvastatin in hyperlipidemic rats on day 8 (Mean ± SD, n=6)

Parameters	Simvastatin + Diltiazem on day 1	Simvastatin + Diltiazem on day 8	Significance
C_{max}(µg/mL)	15.64 ± 0.29	27.45 ± 0.007***	YES
T_{max}(Hrs)	2.00 ± 0.00	2.00 ± 0.00	NS
AUC(tot)	60.19 ± 2.24	78.4 ± 0.80***	YES
$T_{1/2}$(Hrs)	2.12 ± 0.12	2.35 ± 0.03	NS
MRT(min)	3.88 ± 0.17	4.2 ± 0.04	NS
CL/f(mL/kg)	133.78 ± 4.92	102.60 ± 1.06***	YES
V_d(mL)	408.5 ± 12.58	349.00 ± 2.49***	YES

*Significant at P<0.05; **Significant at P<0.01; ***significant at P<0.001 compared to Simvastatin alone day 1 (One way ANOVA followed by Bonferroni's test).

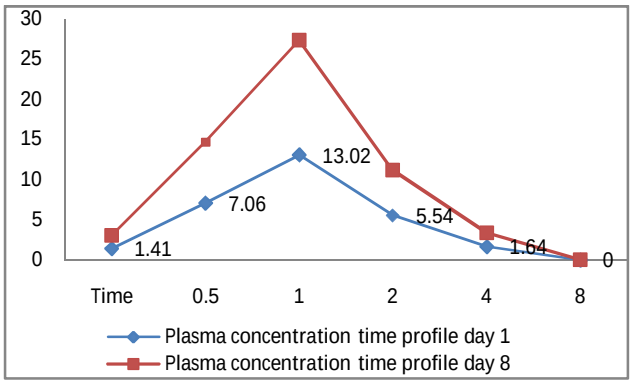

Figure 4: Plasma concentration-time profile of Simvastatin alone on day 1 and with Simvastatin on day 8 (n=6).

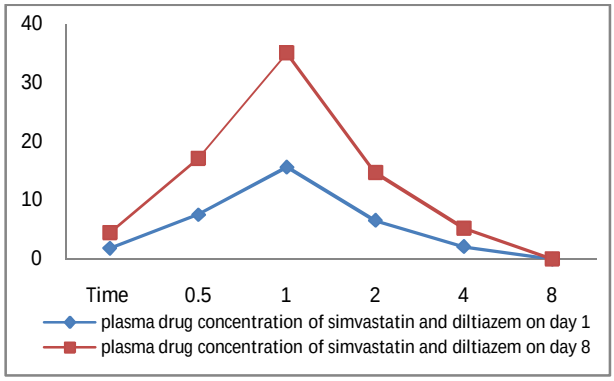

Figure 5: Plasma concentration-time profile of Simvastatin combination with Diltiazem on day 1 and Simvastatin combination with Diltiazem on day 8 (n=6).

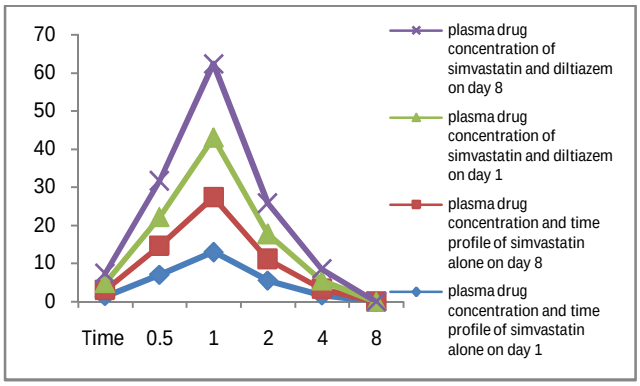

Figure 6: Plasma concentration-time profile of Simvastatin alone on day 1 and Simvastatin alone on day 8 and Simvastatin combination with Diltiazem on day 1 and Simvastatin combination with Diltiazem on day 8 (n=6).

CONCLUSION

This study is to show the bi-directional pharmacokinetic and pharmacodynamic interaction between Diltiazem and Simvastatin after long term treatment with both drugs combined treatment with Diltiazem and Simvastatin increases the C_{max} and AUC of HMG-CoA reductase inhibitor and further reduces total and LDL-cholesterol levels. On the other hand, the combination decreases the C_{max} and AUC of Diltiazem without affecting its blood pressure-lowering effect. These interactions should

therefore be taken into consideration and pharmacokinetic and pharmacodynamic monitoring may be necessary when these drugs are used concomitantly.

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Source of Support: Nil, **Conflict of Interest:** None.