SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND NIFEDIPINE IN RAT PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND PHARMACOKINETIC STUDIES

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Summary

To develop a simple and sensitive high performance liquid chromatographic method for the simultaneous estimation of simvastatin (lipid lowering agent) and nifedipine (calcium channel blocker) in rat plasma and also to calculate possible pharmacokinetic parameters of these drugs after oral administration in hyperlipidemic condition. The plasma samples were precipitated with methanol and after centrifugation (5000-6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. The mobile phase, acetonitrile and water (70:30 v/v), was run isocratically through a C18 analytical column. The UV detection was done at 237 nm and run time was less than 15 minutes. The good linearity was found to be in the range of 0.05 to 40 µg /ml for two drugs, simvastatin and nifedipine, with r^2 value of 0.999. The accuracies for intra-day and inter-day precision were ranged from 94.0% to 98.9% over the concentration range of 0.1 to 40 µg /ml of two drugs. This developed method was rapid, sensitive, reproducible and successfully applied to the measurement of simvastatin and nifedipine in rat plasma for studying of pharmacokinetic interaction between these two drugs.

Key words: Simvastatin, Nifedipine, HPLC method

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Introduction

Simvastatin (S) chemically is (1S,3R,7S,8S, 8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2(2R,4R)Tetrahydro-4-hydroxy-6-OXO-2H-Pyran-2– y1) ethyl] – 1 napthalenyl 2,2 –dimethyl butyrate , a synthetic lipid-lowering agent. It is a selective competitive inhibitor of the enzyme HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate-limiting step in cholesterol biosynthesis [1]. It is used in the treatment of hyperlipidemia. Several methods for its estimation using HPLC [2-3] were reported. Nifedipine (N) is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester, $C_{17}H_{18}N_2O_6$, a dihydropyridine calcium channel blocker [4], used for the treatment of mild to moderate hypertension. A number of HPLC [5-6] methods were reported for estimation of nifedipine, both individually as well as in combination with other drugs except simvastatin. So far no reports are available for the simultaneous estimation of both drugs in rat plasma. To study the pharmacokinetic interaction between these two drugs in hyperlipidemic rats, we developed the new, simple method for simultaneous estimation of simvastatin and nifedipine in rat plasma. The present method is easy and suitable for pharmacokinetic studies of two drugs. Here we also reported the pharmacokinetics of two drugs after oral administration in hyperlipidemic rats.

Materials and Methods

Simvastatin (Orchid Pvt. ltd India) and nifedipine (Nicholus Piramils, India) were obtained as gift samples. Acetonitrile, water (HPLC grade) and ammonium acetate (AR) were purchased from Qualigens Fine chemical (Mumbai, India).

Preparation of stock and standard solutions

Transferred 4 ml of each standard stock solution (2.5 mg/ml in acetonitrile) of simvastatin and standard stock solution (2.5 mg/ml in acetonitrile) of nifedipine in 10 ml of volumetric flask, volume made upto the mark with MilliQ water to get 1 mg/ml. Different working standard solutions were prepared from stock solution by sequential dilution with a combination of acetonitrile: water in 50:50 ratio.

Chromatographic systems and conditions*:*

Shimadzu high performance liquid chromatography unit equipped with the LC-8A Solvent delivery module, SPD-10AVP UV-Visible spectrophotometer detector, Class CR-10 Data Processor, Rheodyne (with 20 µl capacity loop) Injection Port and Wakosil II C-18 Column (stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5µ diameter, 100 A^o pore diameter) were used for analysis of samples. The mobile phase consisted of acetonitrile and water in a combination of 70:30 v/v. Before use the mobile phase was degassed by passing it through a 0.22 µm filter. The mobile phase was pumped at an isocratic flow rate of 1.2 ml/min at room temperature. The UV-detection wavelength was set at 237 nm and sensitivity of 0.001 a.u.f.s was used for the analysis.

Extraction procedure

Rat plasma samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred to a clean tube. The precipitate was resuspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000-6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µl of mobile phase. 20 µl was injected into HPLC system for 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 0.05, 0.1, 0.5, 1, 5, 10, 20, 40 µg/ml. To 50 µl of these solutions, 450 µl of blank plasma and 10 µl of internal standard (IS) ibuprofen (40 µg / ml) were added and vortexed for 2 minute. An equal volume of methanol was added to serum samples for protein precipitation and remaining extraction procedure was described above. The peak areas of simvastatin, nifedipine and ibuprofen were calculated. The peak area ratios obtained for different concentrations of the simvastatin and nifedipine were plotted against the concentration of two drugs. The slope of the plots determined by the method of least square regression analysis (r^2=0.999) was used to calculate the simvastatin and nifedipine concentration in the unknown sample.

Accuracy and precision

Intra day reproducibility was tested by using different concentrations (0.1, 1, 10 and 40 µg/ml). The procedure was repeated on three separate days to allow determination of inter-day precision and accuracy. Intra-day accuracy was estimated based on the mean % error and inter-day accuracy was calculated as the mean of the intra-day 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 (unextracted) in mobile phase to that of samples containing the same amounts of two drugs in plasma after extraction.

Study design

Wistar albino adult male rats weighing 200-230g 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. At the end of the one month the blood

was withdrawn from tail vein to analyze for lipid profiles (TC, TG, LDL-C and HDL-C levels) to confirm the induction of hyperlipidemia.
The hyperlipidemic rats were divided into three groups of six rats in each group.
Group I**:** (HL) Control group of Hyperlipidemic rats received a dose of 1.5% CMC.
Group II**:** (S) simvastatin (80 mg/kg) alone in Hyperlipidemic (HL) rats.
Group III**:** (N) nifedipine (20 mg/kg) alone in HL rats
Group IV**:** (AT-N) simvastatin in combination with nifedipine (20 mg/kg).
This study was carried out for 7 days and the protocol of the present study was approved by the institutional animal ethical committee, Vel's College of Pharmacy, Chennai. India.

Collection of Blood samples

After administration of the drugs blood samples of 0.5 ml were drawn at 0, 0.5, 1, 2, 4, 6, 8 and 24 hrs through retro-orbital sinus into heparinized eppendorff tubes and equal amount of saline were administered to replace blood volume at every blood withdrawal time. The plasma was obtained by immediate centrifugation at 3000 rpm for 5 minutes at room temperature. All samples were stored at 4°C until analysis.

Analysis of study samples:

Study samples were obtained from rats, which were treated with simvastatin and nifedipine since one week. The protocol was approved by institutional animal ethical committee. Six blood samples were collected at 0.5, 1, 2, 4, 8 and 24 hr post oral dose of drugs administration. Plasma was separated and stored at -4^{o} C until analysis.

Statistical analysis

Time to reach peak concentration (t_{max}) was directly taken from observed data. Area under the curve [AUC], elimination half life [$t_{1/2}$], volume of distribution [V/f], total clearance [CL/f], peak plasma concentrations (C_{max}) were calculated for each subject using a non compartmental pharmacokinetic model "WIN NONLIN".

Results and Discussion

Typical chromatogram for simultaneous estimation of simvastatin and nifedipine in rat plasma was shown in fig.1. The retentions times of simvastatin, nifedipine and ibuprofen (internal standard) were approximately 2.4, 8.6 and 19.8 minutes respectively. The analytical run time was 25 min for each plasma sample. The mean extraction efficiencies of simvastatin and nifedipine from serum at a concentration of 0.05-40 µg/ml were 88-93%.

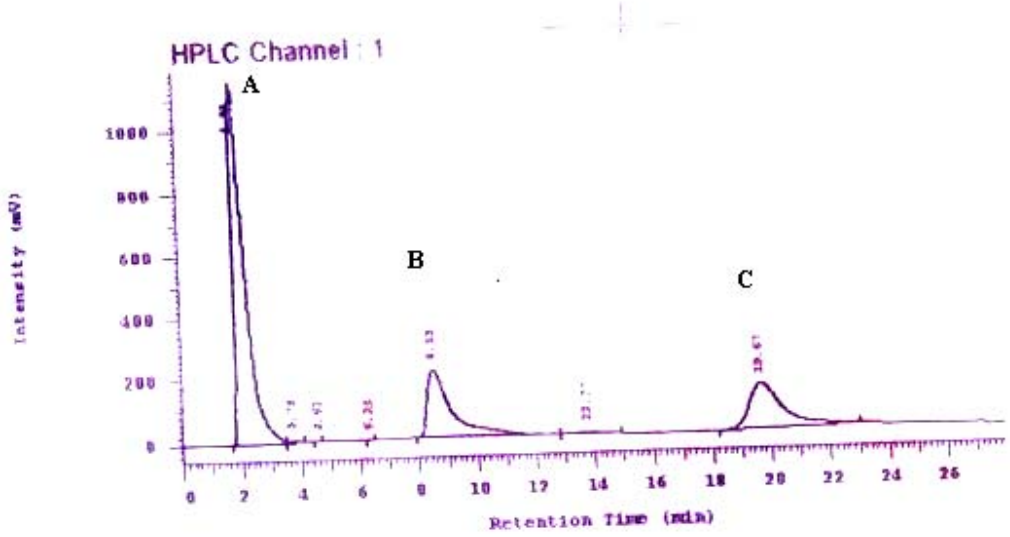

Figure 1

A= Nifedipine, B= Ibuprofen, C= Simvastatin

Method validation

The regression equation for simvastatin and nifedipine were Y = 178329 X +11232 with r^2 =0.9998 and Y= 52411 X + 2675.3 with r^2 =0.9994 (Y=peak area ratio and X=concentration) respectively. The linearity was found to be in the range of 0.05 to 40 µg /ml for two drugs. The calibration curves passes through the origin, which justifies the use of single point calibration. Over the range of concentrations from 0.05 to 40 µg /ml, the intraday accuracies ranged from 94% to 98.6% and 94% to 98.8% for simvastatin and nifedipine respectively (shown in table 1). The average inter-day accuracy were ranged from 94.8% to 98.8% and 95.5% to 98.9% for S and N respectively. The HPLC method described here was accurate and capable of determining concentrations of simvastatin and nifedipine in small volumes of rat serum. The extraction procedure was simple and procedure used a easily available internal standard ibuprofen.

TABLE 1: INTRADAY AND INTERDAY PRECISION AND ACCURACY DATA

Drug	Added Concentration (µg /ml)	Intra-day (n=5)		Inter-day (n=5)	
		Observed Conc. (µg /ml)	Accuracy %	Observed Conc. (µg /ml)	Accuracy %
Simvastatin	0.1	0.094	94.0	0.0948	94.8
	1.5	1.45	96.67	1.44	96.0
	10	9.79	98.4	9.80	98.0
	20	19.72	98.6	19.69	98.45
	40	39.52	98.8	39.55	98.88
Nifedipine	0.1	0.094	94.0	0.095	95.0
	1.5	1.463	97.53	1.45	96.67
	10	9.83	98.3	9.8	98.0
	20	19.73	98.65	19.71	98.55
	40	39.52	98.8	39.56	98.9

Pharmacokinetics

The mean plasma concentration –time curves after oral administration of simvastatin and nifedipine in S and N groups (on day 8) were shown in figure 2 and 3 and corresponding pharmacokinetics were shown in table 2. There is no statistically significant pharmacokinetics interaction ($p>0.005$) was observed between these two drugs in hyperlipidemic condition.

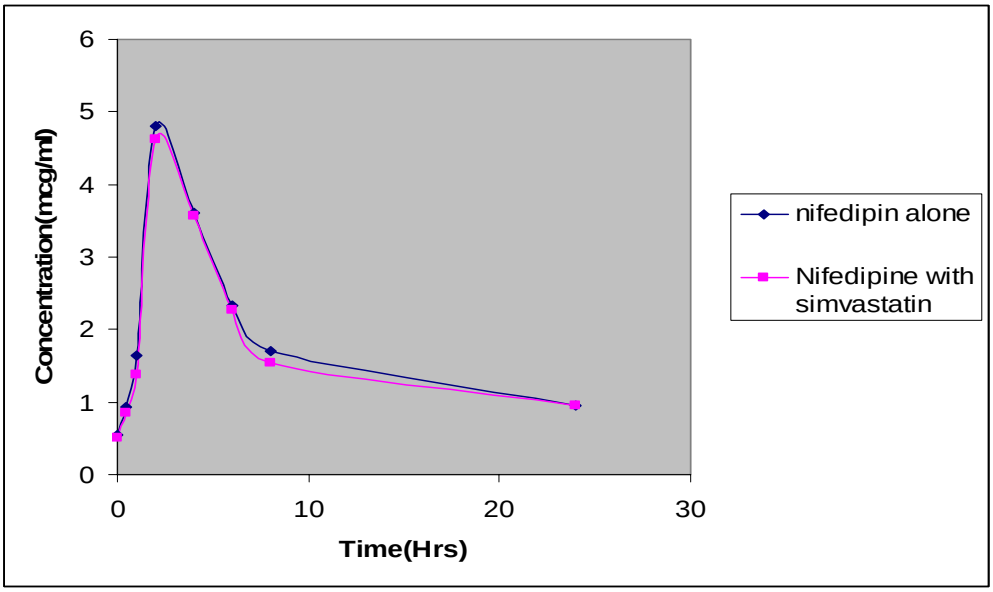

Figure 2: Mean plasma concentrations (µg/ml) of Nifedipine alone and in combination with Simvastatin on day 8 (n=6)

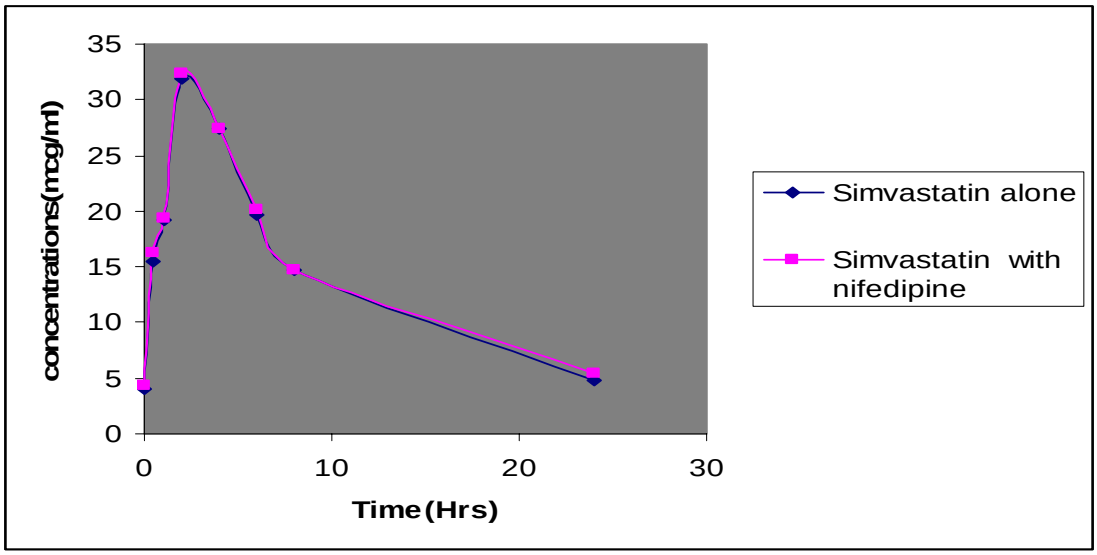

Figure 3: Mean Plasma concentrations (µg/ml) of Simvastatin alone and in combination with Nifedipine on day 8 (n=6)

Table 2: Mean ± SD, pharmacokinetic parameters of simvastatin and Nifedipine in hyperlipidemic rats on day 8

Parameter	Simvastatin alone	Nifedipine alone
Cmax (μg/ml)	31.92±0.22	3.92±0.03
Tmax(h)	2	2
AUCo-t (μg/ml/h)	337.36±0.38	33.49±0.016
AUCo-inf (μg/ml/h)	396.36±0.63	52.33±1.37
T1/2(h)	8.496±0.024	15.22±0.49
CL/f (ml/h/kg)	1.961±0.009	15.475±0.40
V/f (ml/kg)	26.67±1.27	359.16±2.233

In conclusion, a simple and accurate RP-HPLC method has been developed for the determination of simvastatin and nifedipine in rat plasma samples. The method has been successfully used for pharmacokinetic studies in rats.

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