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Research Article

Comparative in vivo study of pure drug and fast dissolving tablets of Simvastatin

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ABSTRACT

The objective of this study was to investigate differences in the pharmacokinetic patterns between pure drug and an optimized formulation of fast dissolving tablets of Simvastatin. The formulations were administered to 2 groups of white New Zealand rabbits (n=6) following cross over design pattern and the plasma levels were measured using LC-MS/MS method. Pharmacokinetic parameters were determined for each formulation. The comparison of the plasma time curves of the dosage forms showed that each dosage form caused significant differences in the drug plasma levels. The highest mean C_{max} value was observed for optimized fast dissolving tablets (68.33 ± 0.42 ng/ml) compared to pure drug (27.72 ± 0.31 ng/ml). The mean time taken to peak plasma concentration for (T_{max}) following administration of pure drug was 11.53 ± 0.01 hours, while it was 6.09 ± 0.072 hour following administration of selected optimized fast dissolving tablets. The elimination rate constant (K_{el}) for pure drug and optimized fast dissolving tablets were found to be 0.58 ± 0.012 h⁻¹ and 0.53 ± 0.014 h⁻¹ respectively. The absorption rate constant (K_a) for pure drug and optimized fast dissolving tablets were found to be 1.68 ± 0.01 h⁻¹ and 5.53 ± 0.02 h⁻¹ respectively. The $AUC_{0-\infty}$ values observed with optimized fast dissolving tablets 686.1 ± 2.07 ng·h/ml in compared to pure drug values 191 ± 1.43 ng·h/ml. Thus, the results of pharmacokinetic studies indicated rapid and higher oral absorption of Simvastatin when administered as its fast dissolving tablets. Both K_a and AUC were markedly increased by fast dissolving tablets.

Keywords: LC-MS/MS, Simvastatin, fast dissolving, In-vivo studies, pharmacokinetic parameters.**Article Info:** Received 07 July 2019; Review Completed 14 August 2019; Accepted 18 August 2019; Available online 30 Aug 2019

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INTRODUCTION

Simvastatin (SIM), a crystalline compound, is practically insoluble in water and hence poorly absorbed from the GI tract. It is a potent and specific inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase, which catalyzes the reduction of HMG CoA to mevalonate. Thus, simvastatin arrests a key step for cholesterol biosynthesis in the liver and is widely used in the treatment of hypercholesterolemia and dyslipidemia as an adjunct to diet. After oral administration, simvastatin is metabolized to its β -dihydroxy acid form (simvastatin acid) by the cytochrome-3A system in the liver, where it inhibits the rate-limiting step in cholesterol biosynthesis. This leads to up-regulation of low-density lipoprotein (LDL) receptors and an increase in catabolism of LDL cholesterol. Being a BCS Class II drug, it often shows dissolution rate-limited oral absorption and high variability in pharmacological effects. The major problem of Simvastatin is its very low water solubility, which results

into poor dissolution rate. The pharmacokinetic performance of Simvastatin fast dissolving tablets was studied in a comparison with that of pure drug in rabbits⁴.

Materials and Methods: The *in vivo* study of the optimized formulations were performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social Justice and Empowerment, Government of India. Prior approval by Institutional animals ethics committee was obtained for conduction of experiments (Ref:VIPER /IAEC/I-7/ 2017-2018, Dated 21-9-2017). Pure Simvastatin and optimized fast dissolving tablets were selected based on *in vitro* release studies and stability conditions were chosen as dosage forms for administration.

Subject selection: The pharmacokinetic performance of Pure Simvastatin and optimized Simvastatin fast dissolving tablets were studied in a randomized crossover study design in rabbits. Twelve New Zealand healthy rabbits with a mean

age of 10 ± 2 weeks and with a mean body weight of 3 ± 0.2 kg were used in this study. Each group consisted of six rabbits ($n=6$) each and were subjected for overnight fasting, it was taken care that there was no stress on the animals. Rabbits were randomly divided into two groups for different sampling time and each group was housed in one cage. Food and water were available ad libitum at all times during the experiment⁷⁻⁸. The study was conducted in a crossover design with 2 weeks washout periods in between the two experiments. The animal dose of Simvastatin was calculated relevant to human dose by using the following formula⁵⁻⁶. The above dosage form was administered through gastric intubation method.

Human dose of Simvastatin = 100 mg.

Animal dose = $\frac{\text{Human dose} \times \text{Animal weight}}{\text{Human weight}}$

$$= \frac{20 \times 3}{70} = 0.85 \text{ mg} = 1 \text{ mg}$$

Blood sampling: About 1 ml of blood samples were collected from the tracheal lobular vein of the rabbit using and the blood was stored in screw top heparinized plastic tubes, the

sampling time for blood was done at 0 (before drug administration), 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 hrs after pure drug administration and at 0, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hrs after administration of Simvastatin fast dissolving tablets. The plasma was immediately separated by aspiration after centrifugation at 4000 rpm for 5 minutes and frozen at -20°C until analyzed by LC-MS/MS method⁷⁻⁸.

Determination of Pharmacokinetic Parameters:

Various pharmacokinetic parameters such as peak plasma concentration (C_{\max}), time at which peak occurred (T_{\max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half-life ($t_{1/2}$) and mean residence time (MRT) were calculated using the noncompartmental pharmacokinetics data analysis software PK Solutions 2.0™ (Summit Research Services, Montrose, CO, USA)⁹. The pharmacokinetic parameters of the tested formulations were statistically analyzed using paired sample's t-test for normal distributed results of C_{\max} , K_a , K_e , MRT and $AUC_{0-\infty}$ value. All tests were performed at 0.001 level of significance¹⁰.

Estimation of Simvastatin in plasma (LC-MS/MS method¹¹):

Chromatographic conditions

A summary of the chromatographic and mass spectrometric conditions is as follows:

UPLC	: WATERS
Mass spectrometer	: API 2000
Ion source	: Heated nebulizer
Polarity	: Negative Detection ions
Simvastatin	: 240.2 * amu (parent), 196.0* amu (product)
d3-Simvastatin	: 244.2* amu (parent), 200.2 amu (product)
Column	: Phenomenax, synergi 4 μ Polar- RP80 A, 4.6x75mm
Column oven temperature	: 35° C
Peltier temperature	: 10° C
Mobile phase	: Ammonium formate: Acetonitrile (30:70)
Flow rate	: 1ml/min.
Volume of injection	: 10 μ L
Retention time	: Simvastatin- 0.5 to 1.20 minutes : ISTD-0.5 to 1.20 minutes
Run time	: 2.00 minutes

Preparation of working standard solutions:

1. Preparation of Simvastatin standard stock solution:

Simvastatin working standard equivalent to 5 mg Simvastatin was accurately weighed and transferred into a 5 ml volumetric flask and dissolved in methanol. The solution was made up to the volume with methanol. The concentration of resulting solution was calculated by considering the purity of Simvastatin. The solutions were labeled and stored in a cold store at $2-8^\circ\text{C}$.

2. Preparation of internal standard stock solution:

0.005 g of d3-Simvastatin was weighed accurately and transferred in to a 5 ml volumetric flask and dissolved in methanol. The solution was made up to the volume with methanol. The concentration of resulting solution was calculated by considering the purity of Simvastatin -d4.

The solutions were labeled and stored in a cold store at $2-8^\circ\text{C}$. Stock solution was diluted with 60% Acetonitrile in water solution to get a concentration of 75.00 $\mu\text{g/ml}$.

Calibration curve standards:**1. Preparation of stock dilutions of standard Simvastatin solution:**

Stock solution of Simvastatin was diluted with 60% Acetonitrile in water solution to get a concentrations ranging from 1 to 140 µg/ml.

2. Spiking of plasma for calibration curve standards:

Concentrations of Simvastatin ranging from 50 to 7000 ng/ml were prepared with plasma and labeled them as CC1 to CC8. The calibration curve standards were prepared freshly for each validation run.

3. Extraction procedure

Step 1: Blank, calibration curve standards and the subject samples were withdrawn from the deep freezer and allowed them to thaw. The thawed samples were vortexed to ensure

complete mixing of contents. To 0.25 ml of plasma sample in a vial, 25 µl of d3-Simvastatin standard (75 µg/ml) was added. To plasma blank, 25 µl of 60% Acetonitrile in water solution was added and vortexed the samples to ensure complete mixing of contents.

Step 2: Add 2.5 ml of tertiary butyl methyl ether, place on a shaker for 15 minutes and centrifuge for 10 minutes at 4000rpm at 20°C. Transfer supernatant (organic layer) into the another vial. Evaporate this layer under a stream of nitrogen at 45 °C. The residue was reconstituted with 0.5 ml of mobile phase and vortexed. The samples were transferred in to auto-injector vials and loaded the vials in to auto sampler. 10 µl of sample was injected in to LC-MS/MS system. Analyte Concentrations of stock dilutions of standard Simvastatin solution with plasma were shown in Table 1.

Table 1: Analyte Concentrations of Stock Dilutions of Standard Simvastatin Solution with Plasma

S.NO	Sample Name	Analyte Concentration (ng/ml)	Analyte peak area	IS Peak Area	Area Ratio	Calculated Concentration (ng/ml)	Accuracy (%)
1	Plasma Blank	0	0	0	0	N/A	N/A
2	Blank+ISTD	0	254	513643	0	N/A	N/A
3	CC1	50.05	3879	528938	0.01	50.407	100.71
4	CC2	100.15	6997	506198	0.01	97.939	97.79
5	CC3	250.300	17284	484351	0.04	258.119	103.12
6	CC4	500.650	34646	504084	0.07	500.215	99.91
7	CC5	1001.250	77371	577631	0.13	978.007	97.68
8	CC6	2002.500	134349	520675	0.26	1887.085	94.24
9	CC7	5006.250	345626	529110	0.65	4782.401	95.53
10	CC8	7008.750	578109	544120	1.06	7780.670	111.01

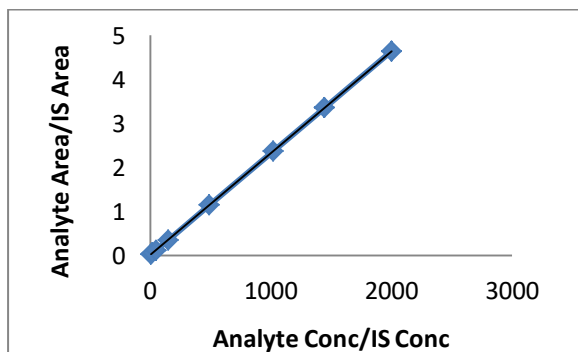


Figure 1: Calibration Curve for Estimation of Simvastatin in Plasma:

4. Data processing

The chromatograms were obtained by using the computer-based Analyst 1.4.2 version software supplied by the Applied Biosystems, Canada. The concentrations of the unknown samples were calculated from the equation using regression analysis of spiked plasma calibration standard with $1/x^2$ as weighting factor. $y = mx + c$; Where, y = Ratio of Simvastatin peak area and ISTD peak area (analyte area / ISTD area); x = Concentration of Simvastatin ; m = Slope of the calibration curve; c = y -axis intercept value. Linear regression analysis equation of stock dilutions of standard Simvastatin solution with plasma is, $y = 0.000136x + 0.000454$.

RESULTS AND DISCUSSION:

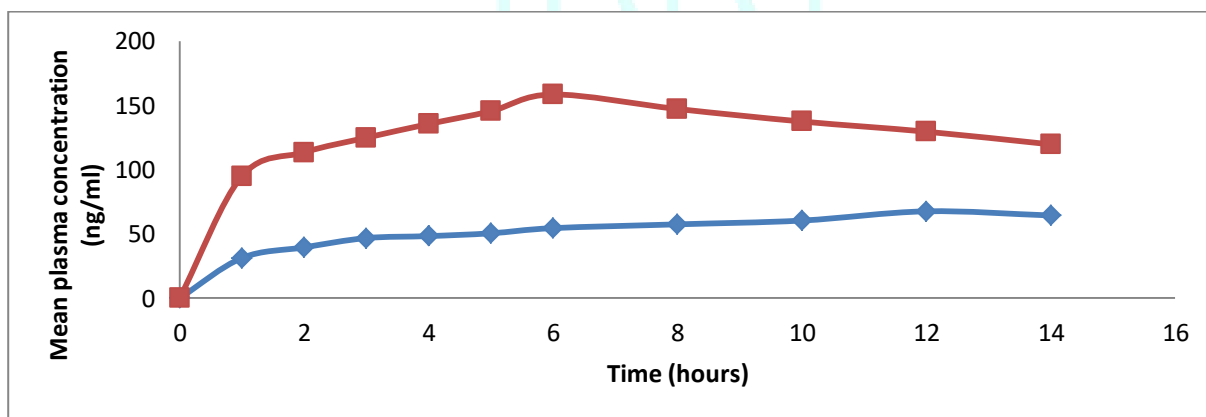
The *in vivo* experiments were conducted as per the protocol and procedure described earlier. Bioanalytical methods employed for the quantitative determination of drugs and their metabolites in biological matrix (plasma, urine, saliva, serum etc) play a significant role in evaluation and interpretation of pharmacokinetic data. For the successful conduct of pharmacokinetic study, the development of selective and sensitive bioanalytical methods plays an important role for the quantitative evaluation of drugs and their metabolites (analytes). The LC-MS/MS methods were

highly sensitive and suitable for the detection of drug in plasma even in low concentrations. Calibration curves were constructed from blank sample (plasma sample processed without IS), blank+IS samples and eight point calibration standards for Simvastatin in plasma. Plasma concentrations of Simvastatin at different times were calculated and are shown in Table 2 and in Fig 2. Pharmacokinetic parameters such as absorption rate constant, elimination rate constant, half-life, AUC, and MRT were calculated from the plot of time versus plasma concentration and subjected to statistical analysis and the results were shown in Table 3.

Table 2: Plasma Concentrations of Simvastatin following oral administration of Pure Simvastatin and optimized fast dissolving tablets

Time (h)	Plasma concentration (ng/ml) (Mean \pm s.d)	
	Pure drug	Simvastatin fast dissolving tablets
0	0	0
0.5	08.51 \pm 1.86	28.23 \pm 1.36
1	11.35 \pm 1.74	36.44 \pm 1.78
1.5	12.51 \pm 1.52	42.20 \pm 1.56
2	14.61 \pm 1.14	45.24 \pm 1.24
3	16.75 \pm 1.64	49.62 \pm 1.12
4	18.81 \pm 1.35	54.12 \pm 1.65
5	19.62 \pm 1.43	61.18 \pm 1.67
6	21.93 \pm 1.24	68.33 \pm 1.85
8	22.76 \pm 1.43	62.15 \pm 1.72
10	24.90 \pm 1.24	57.42 \pm 1.22
12	27.72 \pm 1.27	49.23 \pm 1.36
14	24.64 \pm 1.36	42.20 \pm 1.43
16	21.96 \pm 1.53	35.24 \pm 1.64
18	18.84 \pm 1.32	28.53 \pm 1.18
20	15.92 \pm 1.21	23.12 \pm 1.62
24	12.28 \pm 1.39	17.18 \pm 1.47

FIGURE 2: Concentration-Time Curve of Simvastatin following oral administration of Pure Simvastatin and optimized fast dissolving tablets



(-♦-) plasma Concentration -Time Curve of Simvastatin following pure drug administration

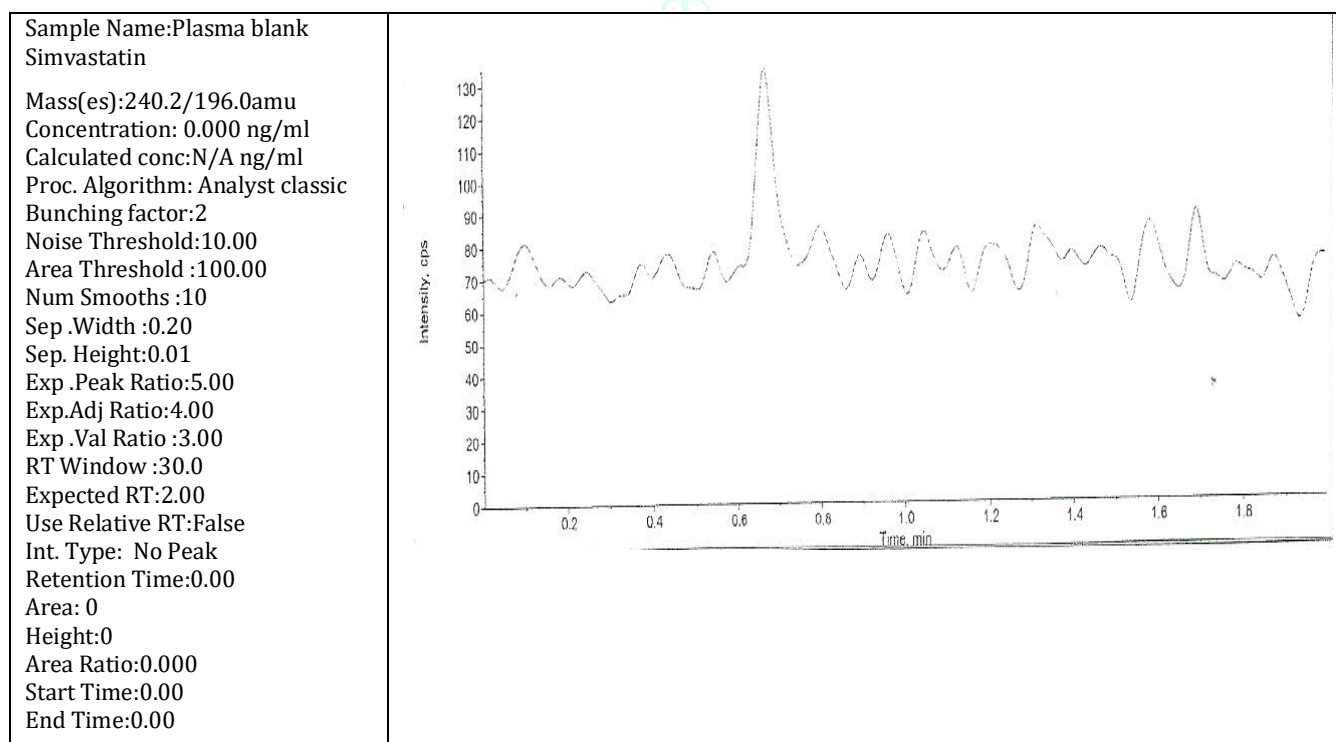
(-■-) plasma Concentration- Time Curve of Simvastatin following optimized fast dissolving tablets administration

Table 3: Statistical Treatment of Pharmacokinetic Parameters (Mean \pm S.D.) of following oral administration of Pure Simvastatin and optimized fast dissolving tablets

Pharmacokinetic parameter	Pure Drug	Optimized fast dissolving tablets	Calculated value of 't'
C_{max} (ng/ml)	27.72 \pm 0.31	68.33 \pm 0.42	26.70***
$t_{1/2}$ (h)	11.53 \pm 0.011	6.09 \pm 0.072	40.75***
K_{el} (h^{-1})	0.58 \pm 0.012	0.53 \pm 0.014	6.87***
K_a (h^{-1})	1.68 \pm 0.01	5.53 \pm 0.02	19.67***
$AUC_{0-\infty}$ (ng h/ml)	191 \pm 1.43	686.1 \pm 2.07	256.60***
Null hypothesis (H_0): There is no significant difference between the pharmacokinetic parameters of Simvastatin obtained with pure drug and optimized fast dissolving tablets. Table value of 't' with 10 DF at the 0.001 level is 4.587.			
Result: H_0 is not accepted as the calculated 't' value more than the table Value of 't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized fast dissolving tablets.			

The results indicated that the parameters significantly differed following optimized fast dissolving tablets administration, compared to pure drug administration. The highest mean C_{max} value was observed for optimized fast dissolving tablets (68.33 \pm 0.42 ng/ml) compared to pure drug (27.72 \pm 0.31 ng/ml). The mean time taken to peak plasma concentration for (T_{max}) following administration of pure drug was 11.53 \pm 0.011 hours, while it was 6.09 \pm 0.072 hour following administration of selected optimized fast dissolving tablets. The elimination rate constant (K_{el}) for pure drug and optimized fast dissolving tablets were found to

be 0.58 \pm 0.012 h^{-1} and 0.53 \pm 0.014 h^{-1} respectively. The absorption rate constant (K_a) for pure drug and optimized fast dissolving tablets were found to be 1.68 \pm 0.01 h^{-1} and 5.53 \pm 0.02 h^{-1} respectively. The $AUC_{0-\infty}$ values observed with optimized fast dissolving tablets 686.1 \pm 2.07 ng hr/ml in compared to pure drug values 191 \pm 1.43 ng hr/ml. Thus, the results of pharmacokinetic studies indicated rapid and higher oral absorption of Simvastatin when administered as its fast dissolving tablets. Both K_a and AUC were markedly increased by fast dissolving tablets.

Figure 3: Chromatograms of reserve stock solution of Standard Simvastatin Solution with Plasma

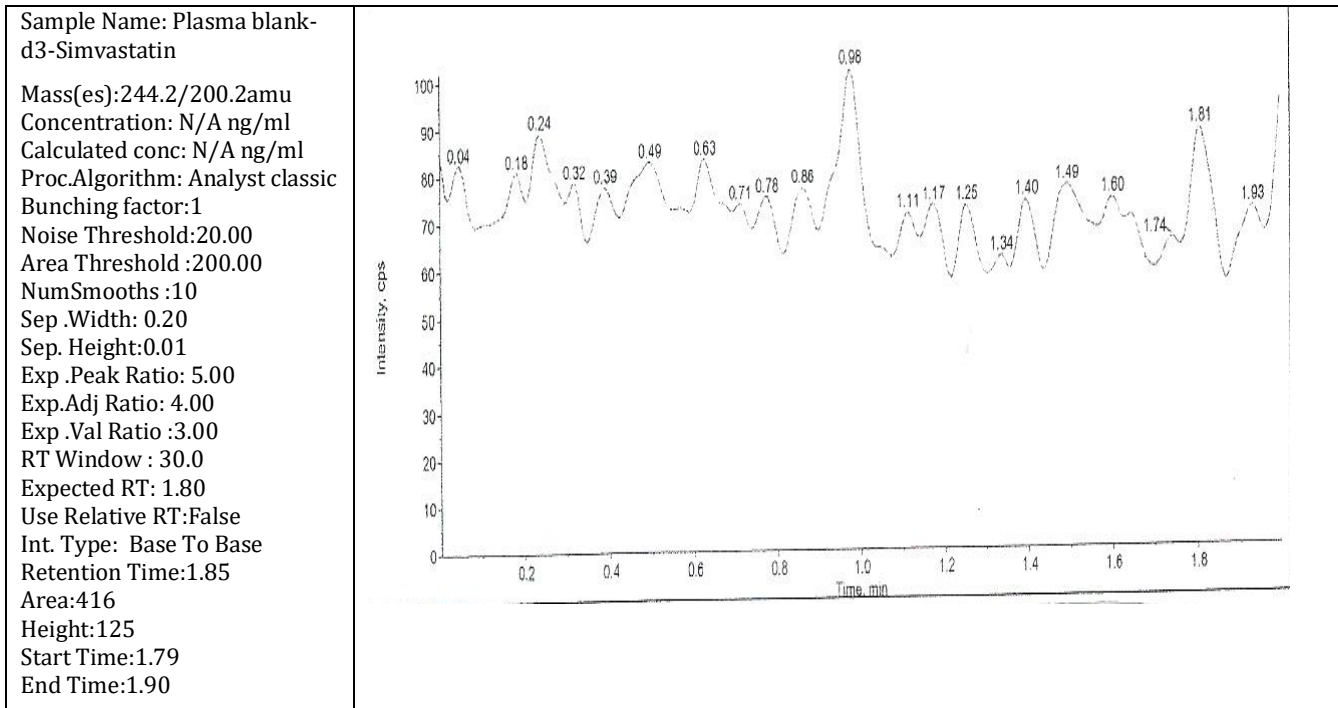
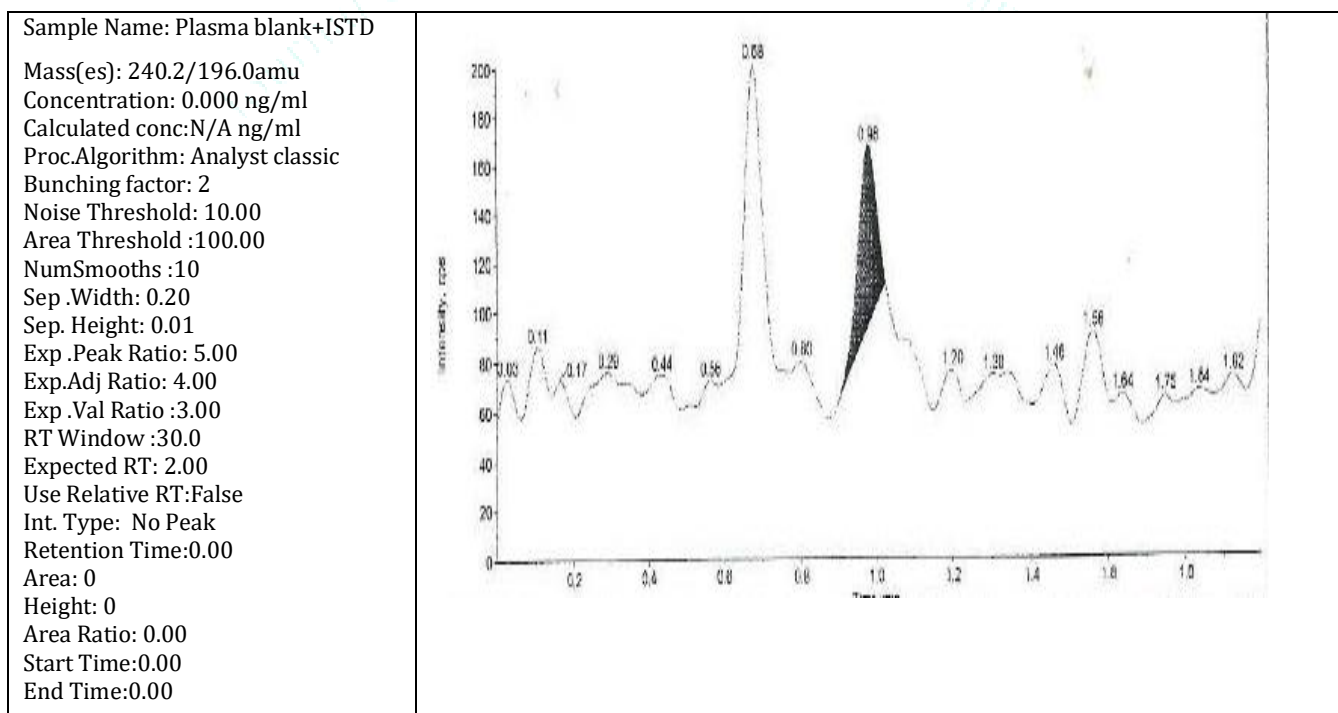
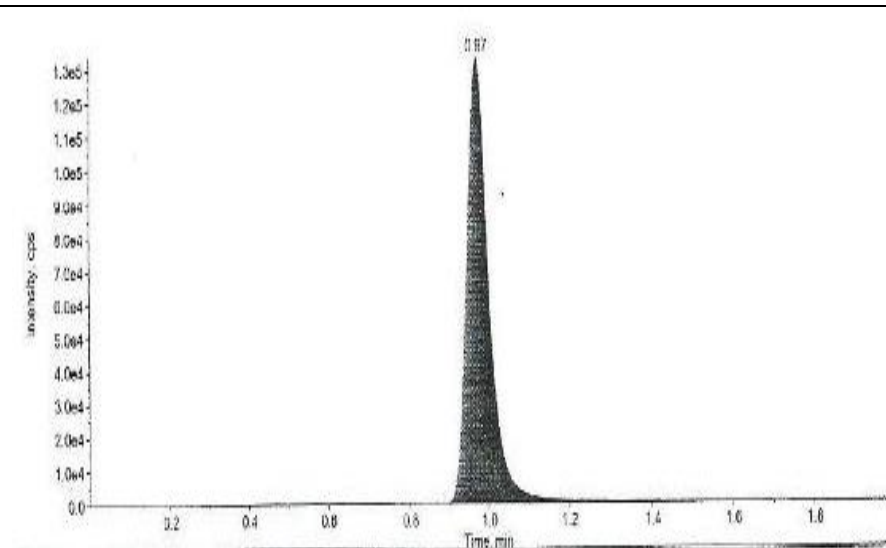


Figure 4: Chromatograms of Plasma blank and internal standard (d3-Simvastatin)



Sample Name: Plasma
 blank+ISTD -d3-Simvastatin
 Mass(es): 244.2/200.2amu
 Concentration: 1.000 ng/ml
 Calculated conc:N/A ng/ml
 Proc.Algorithm: Analyst
 classic
 Bunching factor:1
 Noise Threshold:20.00
 Area Threshold :200.00
 NumSmooths :10
 Sep .Width:0.20
 Sep. Height:0.01
 Exp .Peak Ratio:5.00
 Exp.Adj Ratio:4.00
 Exp .Val Ratio :3.00
 RT Window :30.0
 Expected RT :0.89
 Use Relative RT:False
 Int. Type: Base To Base
 Retention Time:0.97
 Area: 513643
 Height: 1.3e5+0.005
 Start Time:0.89
 End Time: 1.03



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