RESEARCH ARTICLE

Designing, Creating, and Assessing a Modified Pulsincap Delivery System for Intestine Targeting of Fluvastatin in Accordance with Circadian Rhythm

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ABSTRACT

The current study's objective remains to create the Pulscap delivery system using an insoluble capsule core that contains fluvastatin sodium microspheres. Eudragit RL 100 polymer fluvastatin sodium microspheres were made using the solvent evaporation method. To reduce the possibility of stomach upset, these microspheres were hand-filled into capsules, plugged with hydrogel plugs, then sealed with ethyl cellulose acetate and dipped in an eight: two (vol/vol) Acetone: ethanol solution by butyl pthalate of (0.75%) and 5% cellulose acetate phthalate solution. Formulated dosage form was assessed for *in-vivo* X-ray imaging studies, *in-vitro* drug release studies, and *in-vivo* imaging studies, pharmacokinetic study of the optimized formulation was conducted in a two-way cross-over design with 6 rabbits in each group and the pharmacokinetic parameters (C_{max}, K_a, K_e, MRT and $AUC_{0-\alpha}$) are measured. A Pharmacodynamics study was also performed on lipid profiles in six groups of rabbits. Optimized formulation (F20) which is prepared with a drug and polymer ratio of 1:3, is selected based on a dissolution study which shows a percentage release of 99.98. ± 0.12% at 17th hour. In the *in-vivo* study, the plasma concentration at 12th hour 53.8 ± 0.02% and at 32nd hour 13.9 ± 0.05%. Pharmacokinetic parameter C_{max} (ng/mL): 53.8 ± 0.41, MRT (h): 20.0 ± 0.14, $t_{1/2}$ (h): 8.52 ± 0.014, K_{el} (h^{-1}) :0.08 ± 0.014, K_a (h^{-1}):0.25 ± 0.02, AUC_{0-a} (ng h/mL): 944.9. ± 4.05. Serum lipid profile in high cholesterol rabbit after treatment with F20 formulation total cholesterol:240.83 ± 19.76, HDL-C:37.33 ± 2.16, triglycerides:318.67 ± 7.74, VLDL-C:63.73 ± 1.55, LDL-C:139.77 ± 18.74. Compared to lovastatin SR release, lovastatin pulsatile release, and fluvastatin SR release, the results imply that the F20 formulation's pulsatile drug delivery has established good drug delivery for medications entering the gastrointestinal tract, and it displays superior pharmacokinetic and pharmacodynamic parameters.

Keywords: Fluvastatin pulsatile drug delivery, *In-vitro*, *In-vivo*, Pharmacokinetic Study, Pharmacodynamics study.

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INTRODUCTION

This nomenclature refers to the study of biological rhythms (Circadian rhythms) and the systems that control them as "chronobiology." The timing it is of the release of medications essential for the treatment or prevention of various illnesses. The study of creating and assessing medication delivery systems that do so at a rate as close to ideal as possible is known as chronopharmaceutics. The pulsatile drug delivery system is a site-specific, time-controlled medication administration system that is a component of ChrDDSs. By identifying the ideal time to administer a medication, chronotherapy aims to increase therapeutic efficacy and tolerance. The pulsincap device, used for pulsatile drug delivery, is made up of a hydrogel plug for closure and an insoluble substance in water-carrying drug formulation.[1] Pulsincap dissolves in stomach acid after consumption, causing the hydrogel plug to expand steadily. When the larger plug was evacuated at a specific time after eating, the drug content was released.[2,3] In an effort to make the pulsincap technology more straightforward, An erodible tablet has been used in place of the intricate synthetic hydrogel polymer, whose ejection was previously regulated by a sliding friction-controlled mechanism (Figure 1).[4,5]

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Hypercholesterolemia is one the major problems and major risk facotr for the development of cardiovascular disease.[6] Coenzyme A (CoA) reductase, 3-hydroxy-3-methylglutaryl-, the majority of hypercholesterolemia cases are treated with inhibitors and statins.[7] HMG CoA reductase remains rate controlling enzyme of the melvanoate pathway that produces cholesterol. Cholesterol production also follows a circadian phase-dependent pattern, suggesting that this activity follows a 24-hour cycle. Cholesterol synthesis peaks in the wee hours of the morning and at night. So, it's better to take cholesterol-lowering medications before bed than during the day.[8-10]

The statin family of medications, including fluvastatin, is used to treat lipid diseases, including excessive cholesterol. Due to its three-hour biological half-life and limited bioavailability (24–29%). As a result of significant first-pass metabolism make it a great choice for a pulsatile drug delivery method.

The current study aims to develop and examine the *in-vivo* pharmacokinetic and pharmacodynamic properties of fluvastatin microspheres in rabbits for use as pulsatile drug delivery, with the drug being able to be administered at night (9 PM) and released after a predetermined time delay (5 hours), with the drug concentration being most pronounced morning (when levels of free cholesterol are highest).

MATERIALS AND METHODS

Materials

Sodium fluvastatin, Eudragit FS30D, Eudragit RL100, lactose, carbopol, sodium carboxy methyl cellulose, HPMC K100, methylcellulose, sodium hydroxide, potassium dihydrogen orthophosphate, acetone, formaldehyde, liquid paraffin, Tween 80, Span 80, petroleum ether, cellulose acetate phthalate, n-dibutyl phthalate, ethanol.

Dosage Forms

Marketed lovastatin or fluvastatin sodium SR formulation, lovastatin pulsatile release formulation.

Equipment

Electronic balance, pH meter, dissolution apparatus, UV-vis Spectrometer, spray dryer, freeze dryer, IR spectrophotometer, DSC, XRD, SEM, LC-MS/MS.

In LC-MS/MS, atorvastatin was used as an internal standard

MRM Parameters

Lovastatin and simvastatin were ionized using an electron spray ionization source, with the ion spraying voltage set to 5500V, the nebulizer current at 3V, the pressure of a gas at 10 psi, and the pressure of collisions at 8 psi. Other parameters included the declustering potential at 33,400 volts, the focusing potential at 41 volts, the entrance potential at 25 volts, and the exit potential at 60,280.

Reagents

Preparation of 0.1% formic acid buffer

In 585 µL of formic acid was added in 500 mL of water, and the mixture was then exposed to sonication. From that solution, 200 mL was taken and adjusted its pH to 6.0 ± 0.1 with liquor ammonia.

Preparation of mobile phase

To 100 mL of the above 0.1% formic acid buffer (pH 6.0 ± 0.1), 450 mL of acetonitrile and 450 mL of methanol were combined and added them well by sonication.

Preparation of reconstitution solution

To 50 mL to the above 0.1% formic acid buffer (pH not adjusted), 225 mL of acetonitrile and 225 mL of methanol were combined and added them well by sonication.

Making the rinsing solution

To 500 mL of water, 250 mL of acetonitrile and 250 mL of methanol were added and mixed well by sonication.

Method

Formulation of fluvastatin sodium microspheres

Fluvastatin microspheres are prepared by emulsion solvent evaporation technique.[11] F17- F32 formulations are prepared based on the SPAN 80, TWEEN 80 and Eudragit RL 100, Eudragit FS30D. An acetone solution of fluvastatin sodium was dissolved in a 1:1 ratio with polymer (Eudragit FS30/Eudragit RL100). 1% surfactant (tween 80/span 80) was added slowly to 100 mL of liquid paraffin containing 1% homogenous drug and polymer solution over 1-hour, with constant stirring. Three to four washes with petroleum ether were performed after the microspheres had been separated by filtration. A desiccate over-fused calcium chloride was used to keep the microspheres dry after 12 hours. Depending on the core: coat ratio, the polymer will vary from 1:1.5, 1:2, and 1:3.

Preparation of cross-linked gelatin capsules[12-17]

It was decided to take about 100 '0' sized hard gelatin capsules. A wire mesh was then placed over the capsules' bodies. The formaldehyde solution was placed in a desiccator with potassium permanganate added to produce formalin vapors after it had been placed in a desiccator. Over the course of 12 hours, the reaction was carried out. In order to complete the reaction between formaldehyde vapor and gelatin, the bodies were removed and dried at 50°C for 30 minutes. Formaldehyde residues were removed from the bodies by drying them at room temperature.

Preparation of hydrogel plug

In a rotary tablet press, HPMC K100, Carbapol, Na CMC, methylcellulose and lactose were compressed into plugs equal to the volume of the capsule body using 7 mm punches and dies.[18]

Preformulation study

Characterization of microspheres were performed by flow properties, percentage yield, particle size, drug entrapment and *in-vitro* dissolution.

Pharmacokinetics study in rabbits

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

Table 1: Treatment groups based on lovastatin and fluvastatin dosages

Treatment parameters	*Lovastatin*		*Fluvastatin sodium*	
	Marketed SR formulation	*Optimized pulsatile formulation*	*Marketed SR formulation*	*Optimized pulsatile formulation*
Dose of drug (mg) for rabbit weighing 3 kg	2.57	2.57	2.57	2.57
Treatment groups	Group I	Group II	Group I	Group II

Twelve healthy rabbits (mean age 10.2 weeks; mean body weight 3.0–0.2 kg) were used in a randomized two-period crossover experiment with a 2-week washout interval to determine pharmacokinetic parameters. During the experiment, there was unlimited access to food and water and the two sets of animals, each having six in its own cage, were used. Animal doses of lovastatin (0.857 mg/Kg) or fluvastatin sodium (1.7 mg/kg) were administered by gastric intubation method. These doses were calculated relevant to the human dose of lovastatin (20 mg) or fluvastatin (40 mg) by using the following formula.

Table 1 shows the classification of the treatment groups based on lovastatin and fluvastatin dosages

Fluvastatin analysis in plasma

Fluvastatin in plasma was analyzed using LC-MS/MS method (HPLC: Agilent series, Mass spectrometer: API 4000 QTRAP, Ion source as heated nebulizer with positive ion mode,).[19] The separation was performed in a flow rate of 1-mL/min through the column 4.6 x 150 mm, 5 Purospher Star RP-18, using an ammonium acetate buffer as the mobile phase (P^H 4.5 ± 0.1) and acetonitrile (20:80). The stock solution of fluvastatin sodium and it was combined to a 60% methanol in water solution, and the absorption was determined using the line equation of the standard plotted throughout the range of 2.018 to 4044.260 ng/mL. The peak curve of drug in each sample was analyzed.

Pharmacodynamic study

In this experiment, albino rabbits weighing between 1.5 and 2 kg were subjected to a constant ambient temperature of 22°C, between 40 and 50 %RH, and a 12–12-hour cycle between light and dark. Two grams of cholesterol dissolved in maize oil were added to 98 grams of rabbit chow to produce hyperlipidemia. This was done over the course of two weeks. Both the standard FVT and the FVT pulsatile release formulations available on the market were taken orally for 14 days.

Each group consists of 6 rabbits, animals of group-I normal pellet diet was given along with vehicle (Corn oil), the animals of group- II were given with cholesterol diet and served as negative control, group III and IV rabbits were treated with fluvastatin sustained release capsules and pulsatile release capsules, respectively, group V and VI rabbits were treated with lovastatin sustained release capsules and pulsatile release capsules, respectively. All the groups except group I were treated with cholesterol-enriched diet for two weeks and the drug administration was initiated from day 1.

There were six groups of animals

Group I consisted of untreated rabbits (controls), while group II included rabbits given a vehicle considered to be negative. Group III included the rabbits given sustained-release fluvastatin pills. Group IV is considered to be fluvastatin pulsatile release capsule-treated rabbits. Group V rabbits were given sustained lovastatin capsules. Group VI rabbits were given lovastatin pulsatile release capsules.

Procedure

After an overnight fast on day 15, blood was taken from each rabbit's marginal ear vein at various intervals (0, 1, 3, 6, 9, 12 hours). We added EDTA at a final concentration of 1-mg/mL to the blood sample to avoid coagulation and lipoprotein oxidation. The plasma was spun at 1500 x g for 10 minutes to separate the different lipoprotein and triglyceride subfractions, then it was kept at 4°C for analysis. To calculate LDL cholesterol and VLDL cholesterol, Friedewald's method was applied:

VLDL cholesterol = Triglyceride/5 and LDL cholesterol = Total cholesterol-(VLDL + HDL cholesterol).

Statistical Analysis

The results of the data's statistical analysis were displayed as a mean and SD. The data were statistically analyzed using a student t-test. The differences were deemed statistically significant at the p 0.05 level and extremely significant at the p 0.001 level.

RESULTS AND DISCUSSION

Preformulation and formulation studies were performed and the results of characterization were analyzed (Figure 1 and 2).[20] From the previous study, F20 formulation was finalized which is used to for further *in-vivo* study.

***In-vivo* Study**

A standard graph and the concentrations of analytes in stock dilutions of the stock fluvastatin plasma and sodium solution were made (Table 2). The plasma concentrations of standard fluvastatin sodium solution stock solutions (Table 3) and fluvastatin sodium solution were evaluated (Table 4). The supplementary has graphs for atorvastatin and fluvastatin was given in Supplementary Figure 1.

The time plots of plasma concentration in rabbits after administration of marketed and test formulations are shown in Tables 5 and 6. The time to achieve maximum concentration of FVT was delayed by 4 hours in FVT microspheres compared to the marketed formulation.

Table 2: Standard fluvastatin sodium solution concentrations of standard dilutions

Standard conc (ng/mL)	*Stock volume (mL)*	*Amount of diluent (mL)*	*Total volume (mL)*	*Total dissolved solids (ng/mL)*
10110.65	0.800	1.200	2.000	4044.26
10110.65	0.400	1.600	2.000	2022.14
4044.3	0.400	1.600	2.000	808.86
4044.3	0.100	1.900	2.000	202.20
808.98	0.100	1.900	2.000	40.440
202. 40	0.100	1.900	2.000	10.120
40.369	0.200	1.800	2.000	4.037
40.369	0.100	1.900	2.000	2.018

Table 3: Standard fluvastatin sodium solution concentrations of stock dilutions with plasma

Concentration of stock (ng/ mL)	*Stock conc (mL)*	*Plasma volume (mL)*	*Final volume (mL)*	*Concentration at final (ng/mL)*	*Labeled*
4044.26	0.050	0.950	1.000	202.213	CC8
2022.14	0.050	0.950	1.000	101.107	CC7
808.86	0.050	0.950	1.000	40.443	CC6
202.20	0.050	0.950	1.000	10.110	CC5
40.440	0.050	0.950	1.000	2.022	CC4
10.120	0.050	0.950	1.000	0.506	CC3
4.037	0.050	0.950	1.000	0.202	CC2
2.018	0.050	0.950	1.000	0.101	CC1

Table 4: Analyte concentrations of standard fluvastatin sodium solution with plasma dilutions of stock

S. No.	*Labeling of the sample*	*Concentration analyte (ng/mL)*	*Peak area obtained for analyte*	*Peak area IS*	*Obtained area ratio*	*Obtained concentration (ng/mL)*	*Accuracy (%)*
1	Aqueous mixture	N/A	2247	1940259	0.001160	0.094	-
2	Blank plasma	0.0	0.0	0.0	0.0	-	-
3	Blank +std	0.0	0.0	1217732	0.0	-	-
4	CC_1	0.101	1325	1102974	0.0012	0.098	97.03
5	CC2	0.202	2696	1132350	0.00238	0.197	97.52
6	CC3	0.506	7223	1258131	0.00574	0.481	95.06
7	CC4	2.022	30281	1277746	0.0237	1.995	98.66
8	CC5	10.11	150940	1281233	0.118	9.933	98.25
9	CC6	40.443	584605	1221929	0.478	40.351	99.77
10	CC7	101.107	1494406	1242395	1.2	101.455	100.34
11	CC8	202.213	2956131	1271509	2.32	196.099	96.98

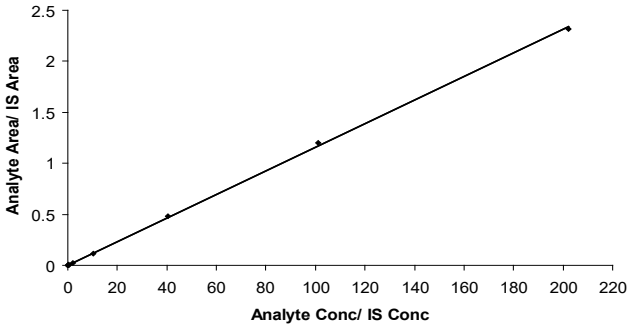

Figure 1: Fluvastatin sodium in plasma estimation calibration curve

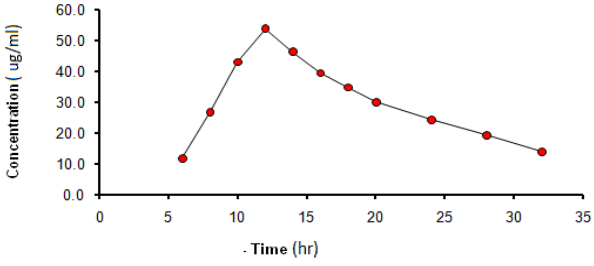

Figure 2: Fluvastatin sodium graph of plasma concentration with time following oral dose of pulsatile formulations

Table 5: Plasma concentration of fluvastatin sodium following oral administration of a marketed formulation

Time in hours	*Plasma fluvastatin sodium market available sr formulation concentration (ng/mL) Mean ± S.D*
0	0.0
0.5	6.12 ± 0.04
1.0	12.34 ± 0.03
2.0	17.89 ± 0.06
4.0	33.12 ± 0.05
6.0	48.59 ± 0.02
8.0	42.45 ± 0.01
10.0	28.59 ± 0.04
12.0	19.34 ± 0.05
16.0	10.59 ± 0.08
20	2.98 ± 0.02
24.0	0.254 ± 0.01
48	0.032 ± 0.07

Table 6: Plasma concentration of fluvastatin sodium following oral administration of pulsatile formulations

Time in hours	*Fluvastatin sodium optimized pulsatile formulation plasma conc. in ng/mL (Mean ± S.D)*
0	0.0
2	0.0
4	0
6	11.67 ± .03
8	26.78 ± 0.02
10	42.89 ± 0.03
12	53.80 ± 0.02
14	46.39 ± 0.04
16	39.27 ± 0.03
18	34.69 ± 0.01
20	29.86 ± 0.02
24	24.21 ± 0.04
28	19.2 ± 0.03
32	13.9 ± 0.05

Table 7: Pharmacokinetic parameters (Mean ± SD) treated statistically after orally administering commercially available sustained release formulation and designed pulsatile formulations of fluvastatin sodium

The parameters involved in pharmacokinetics	*Commercialized SR Remedy*	*Designed pulsatile form*	*"t" value obtained*
C_{max} (nanogm/mL)	47.3 ± 0.24	53.8 ± 0.41	13.50***
MRT in hours	14.4 ± 0.12	20 ± 0.14	23.40***
$t_{1/2}$ (h)	6.19 ± 0.03	8.52 ± 0.014	6. 45***
Kel /h	0.11 ± 0.08	0.08 ± 0.014	2.67***
Ka/h	0.39 ± 0.07	0.25 ± 0.02	14.45***
$_{AUC0-a}$(ng h/mL)	403.1 ± 1.22	944.9. ± 4.05	137.56***

Null hypothesis (H_o): Pharmacokinetic characteristics following oral ingestion of commercially available SR and pulsatile formulations of Fluvastatin sodium are similar. With 10 DF at the 0.001 level, the value of 't' in the table is 4.587.

When compared to the value of 't' in the table with 10 DF at significance stages at 0.001, the calculated value of Ho for t is rejected. Pharmacokinetic characteristics of the use of pulsatile formulations Fluvastatin sodium were found to differ significantly then compared to be commercially available; this led to the conclusion that there was a difference between the two.

Values were represented n = 6 as Mean SD, * p 0.05, ** p 0.01 and *** p 0.001

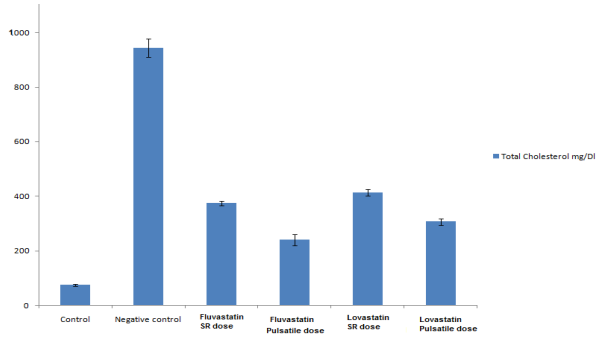

Figure 3: Analysis of TC levels in 6 groups

Table 8: High-cholesterol diet's effects on serum lipid levels

Groups	*TC*	*HDLC*	*Triglycerides*	*VLDLC*	*LDLC*
Control	74.67 ± 3.72	33.00 ± 5.06	106.83 ± 6.85	21.37 ± 1.37	19.30 ± 3.46
Negative control	943.33 ± 36.5	217.67 ± 3.83	989.50 ± 25.22	197.90 ± 5.04	727.77 ± 39.89
Fluvastatin SR release	374.50 ± 7.58	25.17 ± 2.79	420.50 ± 11.29	84.10 ± 2.26	265.23 ± 9.6
Fluvastatin pulsatile release	240.83 ± 19.76	37.33 ± 2.16	318.67 ± 7.74	63.73 ± 1.55	139.77 ± 18.74
Lovastatin SR release	413.83 ± 11.79	24.50 ± 3.51	367.67 ± 10.67	73.53 ± 2.13	315.80 ± 15.86
Lovastatin pulsatile release	306.17 ± 11.55	31.33 ± 3.27	337.50 ± 11.02	67.50 ± 2.2	207.33 ± 10.06

Values expressed are mean ± SEM of six observations, values of control, fluvastatin and lovastatin are associated by negative utilizing Dunnett's test along with one-way ANOVA as a control. *p <0.05, **p <0.01, * p <0.001, ns = non significant

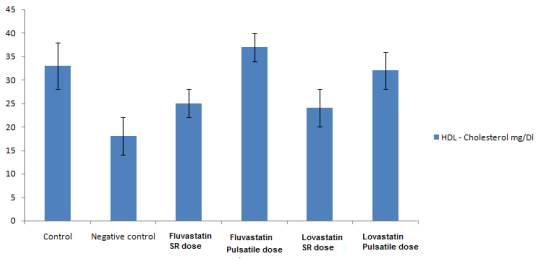

Figure 4: Comparison of HDL C in six groups of animals

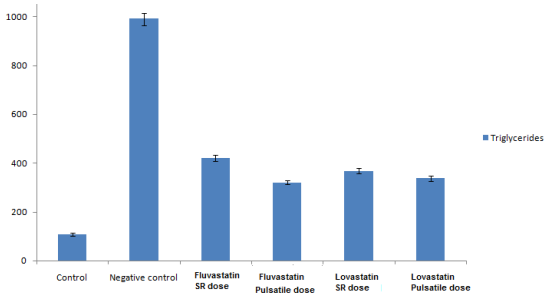

Figure 5: Analysis of triglycerides levels in six groups

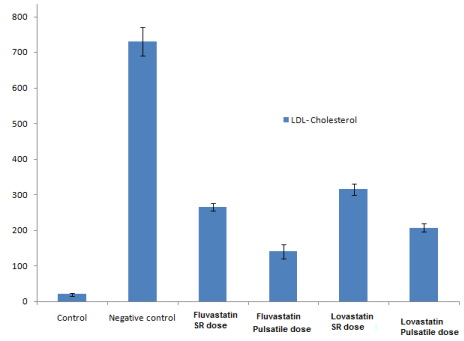

Figure 6: Comparison of LDL-C In six groups of rabbits

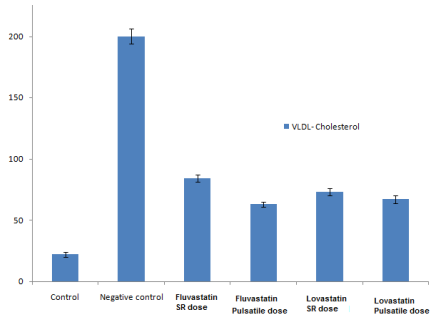

Figure 7: Comparison of VLDL-C in six groups of animals

The results of pharmacokinetics were tabulated in Table 7. The t_{max} was achieved at 12th hours t1/2 was 8.52 ± 0.0014 hours and the C_{max} was 53.8 ± 0.41 (ng/mL) after administration of FVT microspheres compared to marketed formulation t_{max} was achieved at 8th hours t1/2 was 6.19 ± 0.03 hours and the C_{max} was 47.3 ± 0.241 (ng/mL). The $AUC_{0-\infty}$ of FVT microspheres was 944.9 hour/mL 23.44 fold higher than $AUC_{0-\infty}$ of marketed FVT was 403.1, clearly displaying the superiority in performance compared to marketed FVT.

Pharmacodynamic Study in Rabbits

Among formulations (Group 3–6) fluvastain lowers the total cholesterol level to 240.83 ± 19.76 from 943.33 ± 36.5, and also the LDL levels to 139.77 ± 18.74, triglyceride reduction to 318.67 ± 7.74 and VLDL-C reduction to 63.73 ± 1.55. Increase in the HDL-C to 37.33 ± 2.16. Table 8 and Figures 3-7 show the values of TC, HDLC, triglycerides, VLDC and LDLC among various groups

CONCLUSION

This study's goal was to investigate the viability of fluvastatin in chronotherapy. FVT pulscap exhibited time-dependent drug release with T_{max} around 12th hour. The pharmacological study shows a reduction in bad cholesterol and an increase in good cholesterol. The development of fluvastatin pulsatile medication delivery and evaluation of it was successful.

NOTE

Our research was conducted in accordance with the CPCSEA guidelines established by the Indian Government's Ministry of Social Justice and Empowerment.

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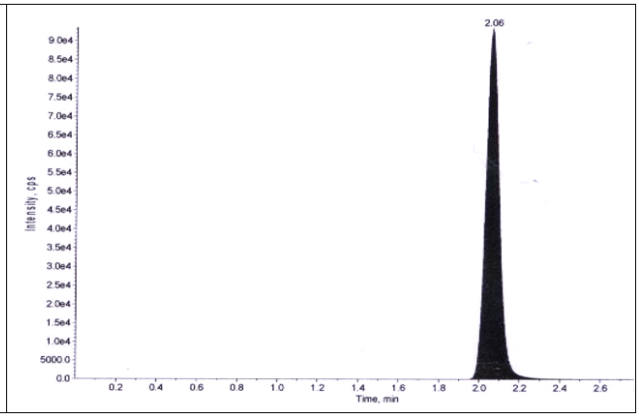
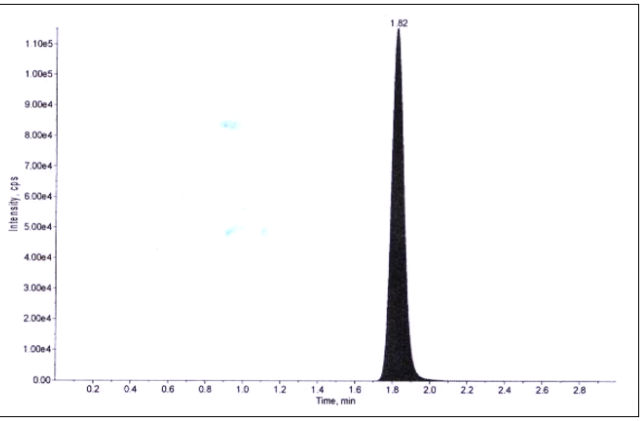
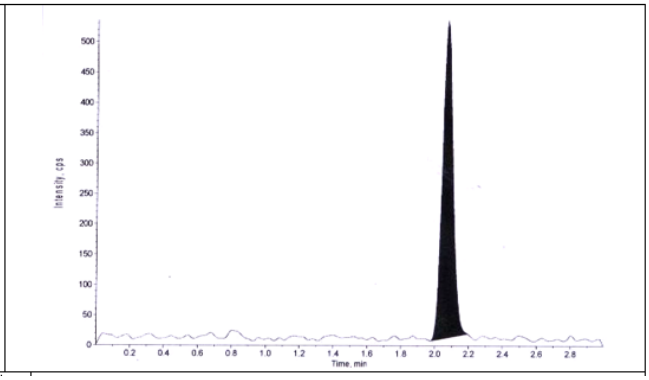
Sample Name: Fluvastatin sodium
10ng/ml+ISTD
10 ng/ml
Mass(es): 415.3/178.0 amu
Concentration: N/A ng/ml
Calculated conc:68.198 ng/ml
Proc.Algorithm: Analyst classic
Bunching factor: 2
Noise Threshold:10.00
Area Threshold :100.00
Num Smooths :10
Sep .Width:0.20
Sep. Height:0.01
Exp .Peak Ratio:5.00
Exp.Adj Ratio:4.00
Exp .Val Ratio :3.00
RT Window :30.0
Expected RT:2.00
Use Relative RT:False
Int. Type: Base To Base
Retention Time:2.06
Area:433160
Height:93400
Area Ratio:0.809000
Start Time:1.93
End Time:2.41

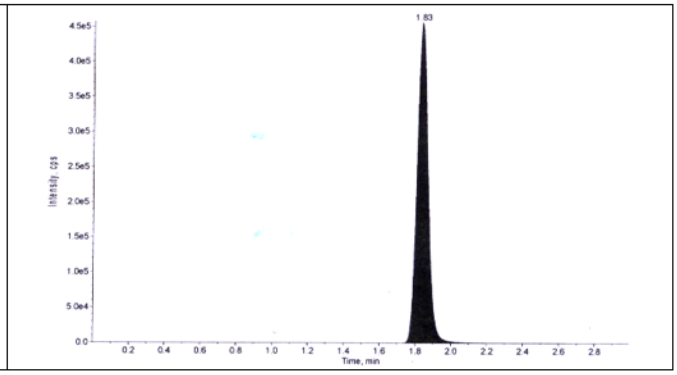
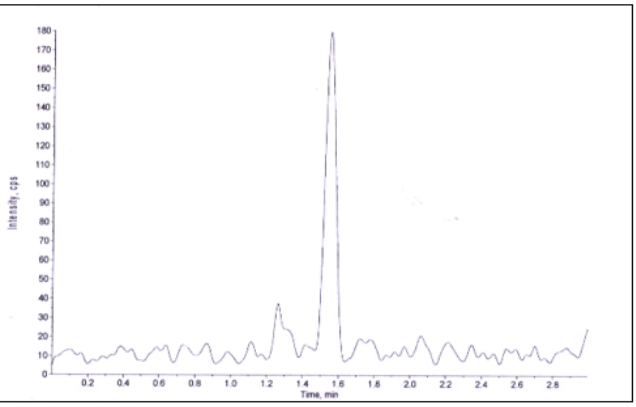
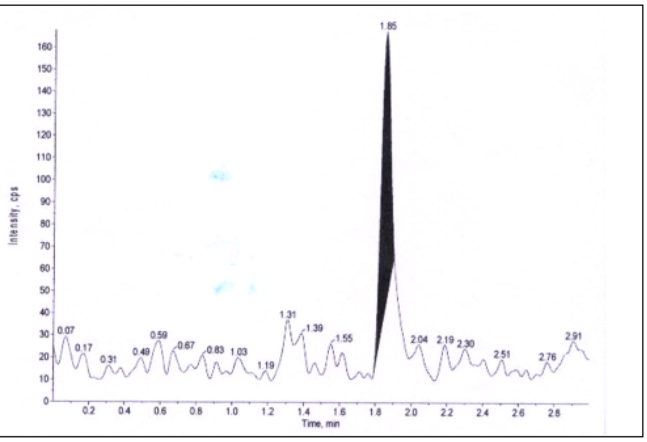
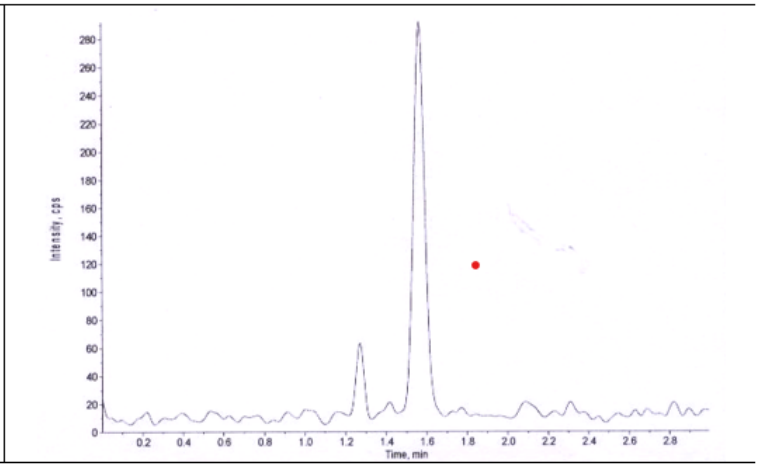


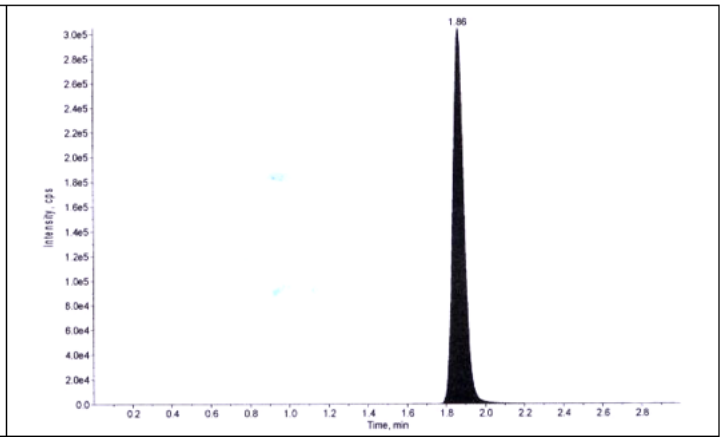
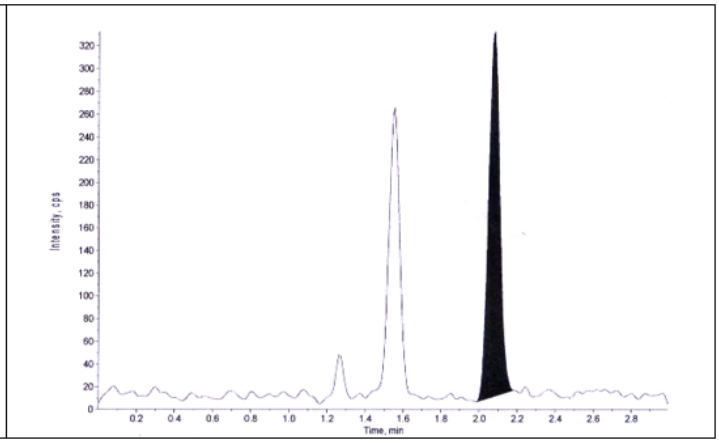
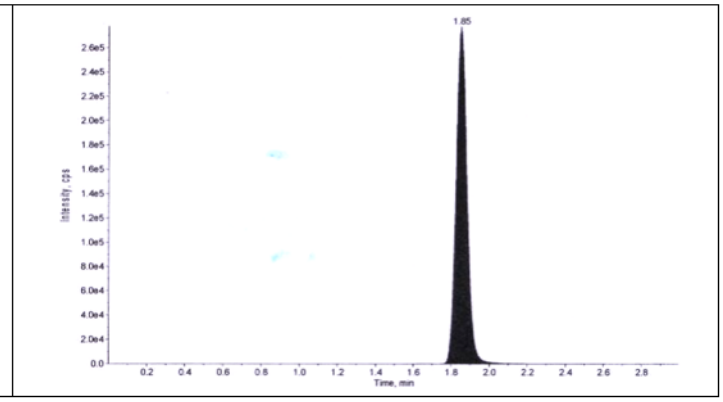
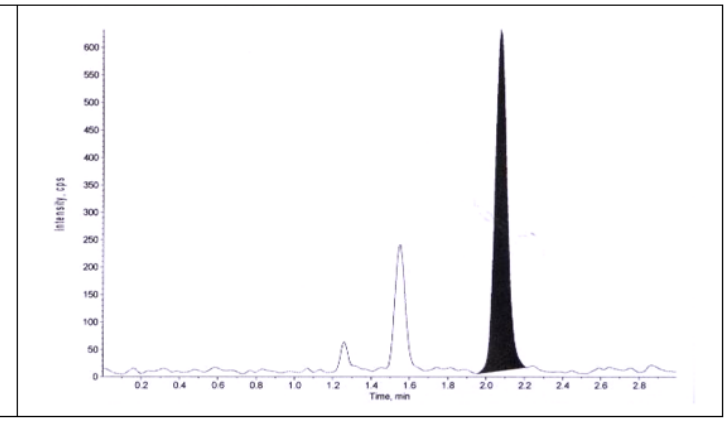
Sample Name: : Atorvastatin
Mass(es): 455.5/165.1
Concentration:1.000 ng/ml
Calculated conc:68.198 ng/ml
Proc.Algorithm: Analyst classic
Bunching factor:1
Noise Threshold:20.00
Area Threshold :200.00
Num Smooths :10
Sep .Width:0.20
Sep. Height:0.01
Exp .Peak Ratio:5.00
Exp.Adj Ratio:4.00
Exp .Val Ratio :3.00
RT Window :30.0
Expected RT:1.80
Use Relative RT:False
Int. Type: Base To Base
Retention Time:1.82
Area:535715
Height:116000
Area Ratio:
Start Time:1.70
End Time:2.08

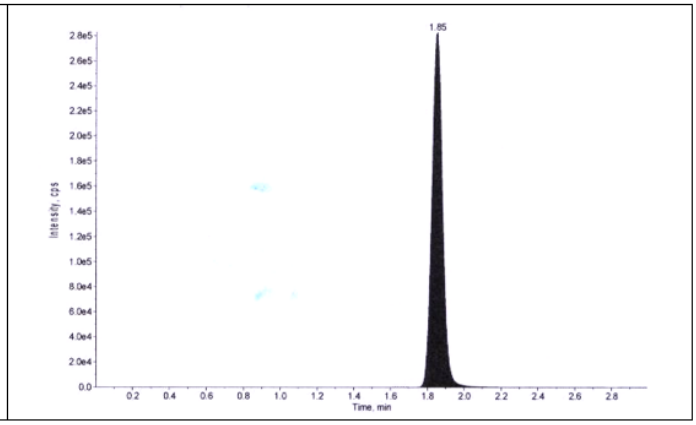
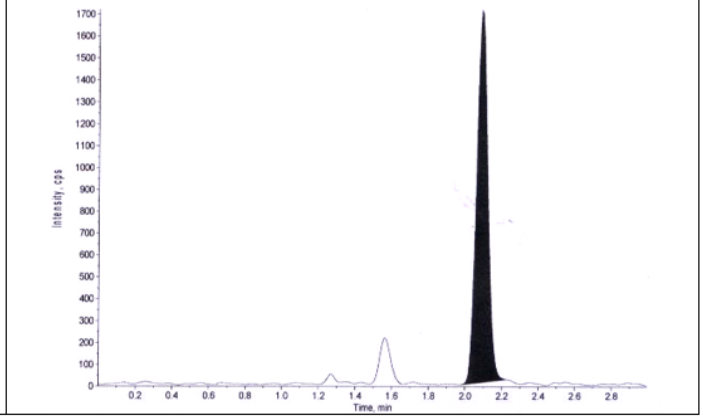
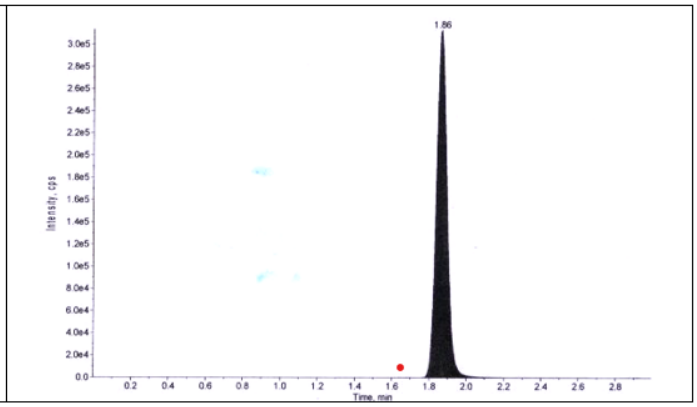
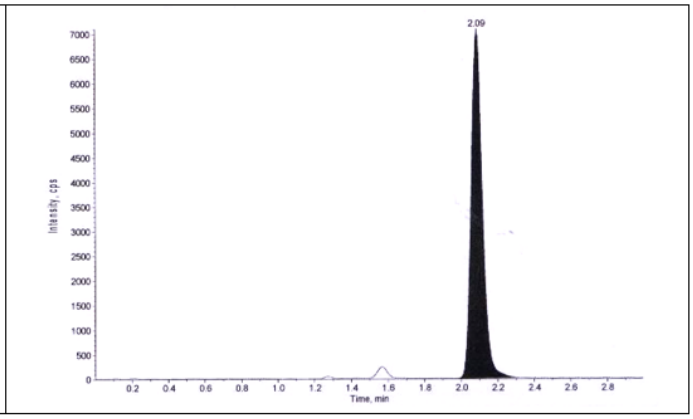


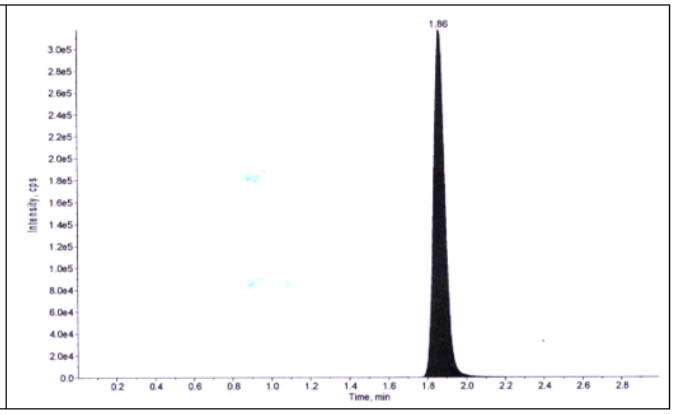
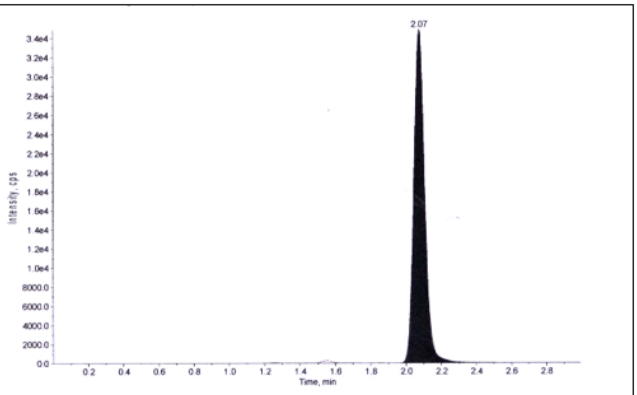
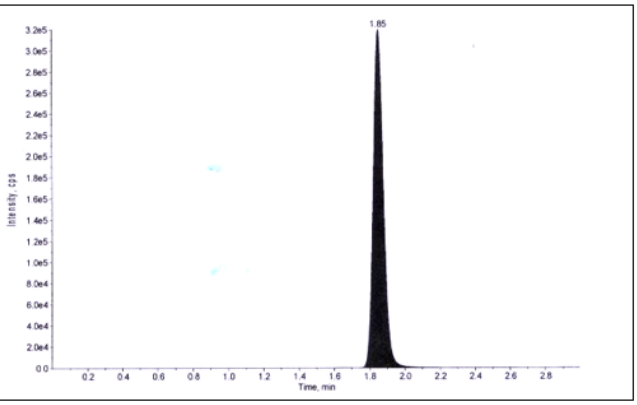
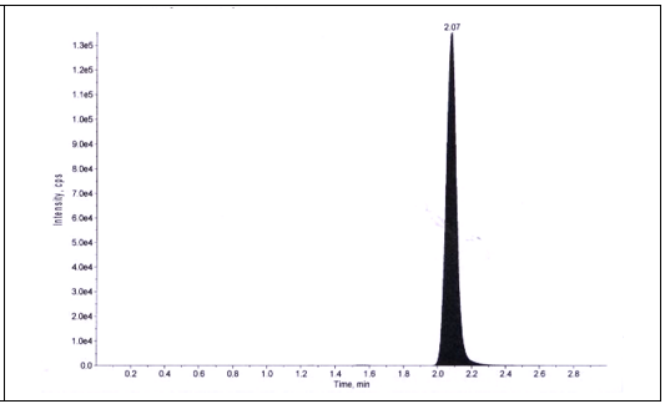
Sample Name: System suitability- Fluvastatin sodium
Mass(es):415.3/178 amu
Concentration: N/A ng/ml
Calculated conc: 0.094 ng/ml
Proc.Algorithm: Analyst classic
Bunching factor: 2
Noise Threshold:10.00
Area Threshold :100.00
Num Smooths :10
Sep .Width:0.20
Sep. Height:0.01
Exp .Peak Ratio:5.00
Exp.Adj Ratio:4.00
Exp .Val Ratio :3.00
RT Window :30.0
Expected RT:2.00
Use Relative RT:False
Int. Type: Base To Base
Retention Time:2.07
Area:2247
Height:523
Area Ratio:0.001160
Start Time:1.98
End Time:2.19

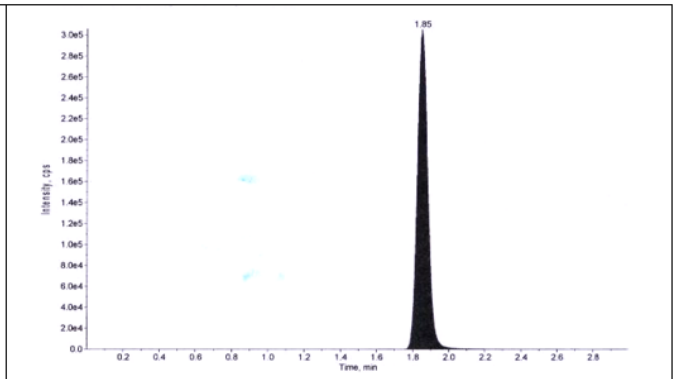
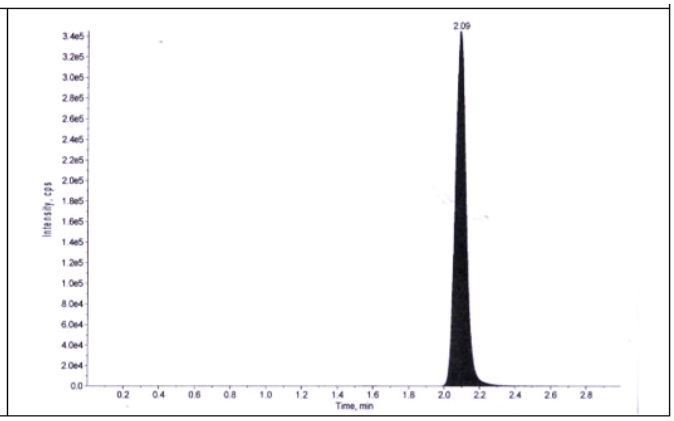
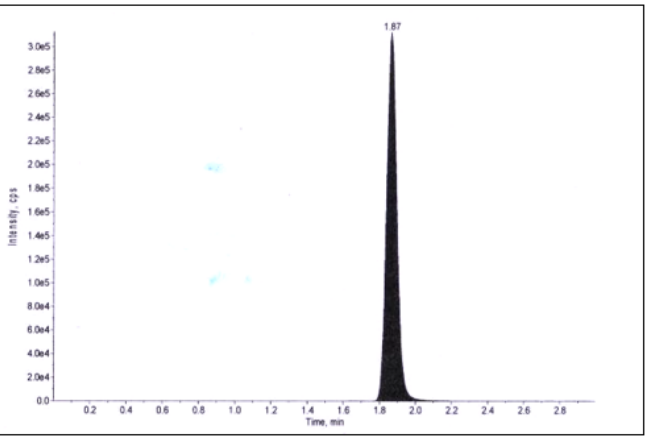
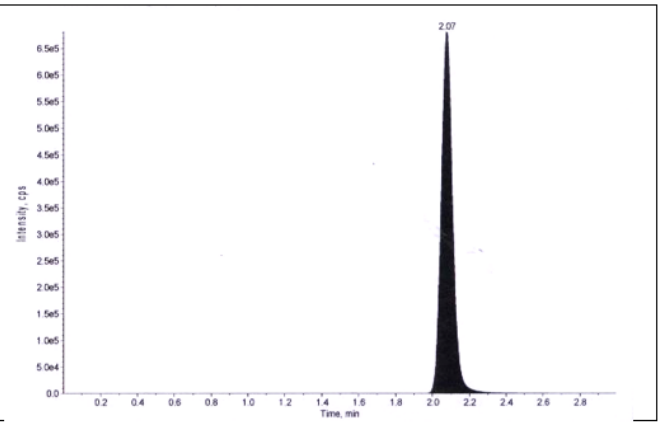


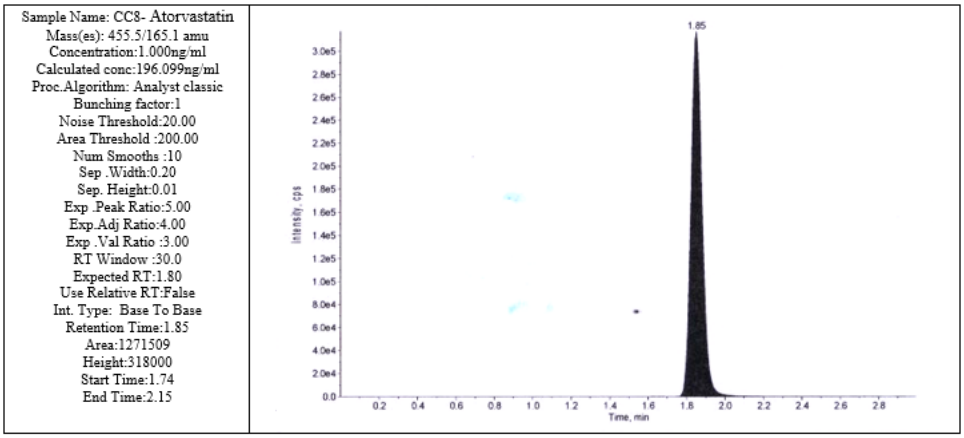
Sample Name: System suitability- : Atorvastatin Mass(es):455.5/165.1amu Concentration: 1.000 ng/ml Calculated conc: 0.094 ng/ml Proc..Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20 Area Threshold :200.00 Num Smooths :10 Sep .Width:0.20 Sep. Height:0.01 Exp .Peak Ratio:5.00 Exp.Adj Ratio:4.00 Exp .Val Ratio :3.00 RT Window :30.0 Expected RT:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:1.83 Area:1940259 Height:458000 Start Time:1.72 End Time:2.17	
Sample Name: Plasma blank- Fluvastatin sodium Mass(es):415.3/178.0amu Concentration: 0.000 ng/ml Calculated conc:N/A ng/ml Proc..Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: No Peak Retention Time:0.00 Area: 0 Height:0 Area Ratio:0.000 Start Time:0.00 End Time:0.00	
Sample Name: Plasma blank- Fluvastatin sodium Mass(es):455.5/165.1 amu Concentration: N/A ng/ml Calculated conc: N/A ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :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: 1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.85 Area:416 Height:125 Start Time:1.79 End Time:1.90	
Sample Name: Plasma blank+ISTD Mass(es):415.3/178 amu Concentration: 0.000 ng/ml Calculated conc:N/A ng/ml Proc..Algorithm: Analyst classic Bunching factor: 2 Noise Threshold: 10.00 Area Threshold :100.00 Num Smooths :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: 2.00 Use Relative RT:False Int. Type: No Peak Retention Time:0.00 Area: 0 Height: 0 Area Ratio: 0.00 Start Time:0.00 End Time:0.00	

Sample Name: Plasma blank+ Atorvastatin Mass(es):455.5/165.1 amu 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 Num Smooths :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 RT1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.86 Area: 1217732 Height: 306000 Start Time:1.76 End Time:2.15	
Sample Name:CC1- Fluvastatin sodium Mass(es):415.3/178 amu Concentration: 0.101 ng/ml Calculated conc:0.098 ng/ml Proc.Algorithm: Analyst classsic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time: 2.08 Area: 1325 Height: 322 Area Ratio:0.001200 Start Time:1.99 End Time:2.18	
Sample Name: CC1- : Atorvastatin Mass(es):455.5/165.1 amu Concentration:1.000 Calculated conc:0.098 Proc.Algorithm: Analyst classsic Bunching factor:1 Noise Threshold: 20.00 Area Threshold : 200.00 Num Smooths :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:1.80 Use Relative RT:False Int. Type: Base To Base Retention Time: 1.85 Area: 1102974 Height:278000 Start Time:1.74 End Time:2.11	
Sample Name:CC2 Fluvastatin sodium Mass(es):415.3/178 amu Concentration:0.202 ng/ml Calculated conc:0.197 ng/ml Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:2.07 Area:2696 Height:622 Area Ratio : 0.002380 Start Time:1.95 End Time:2.21	

Sample Name: CC2 -- : Atorvastatin Mass(es):455.5/165.1 amu Concentration:1.000 ng/ml Calculated conc:0.197 ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold: 20.00 Area Threshold :200.0 Num Smooths :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:1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.85 Area:1132350 Height:284000 Start Time:1.74 End Time:2.13	
Sample Name:CC3- Fluvastatin sodium Mass(es): 415.3/178 amu Concentration:0.506 ng/ml Calculated conc:0.481ng/ml Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:2.09 Area:7223 Height:1710 Area Ratio: 0.005740 Start Time:1.98 End Time:2.22	
Sample Name: CC3- : Atorvastatin Mass(es): 455.5/165.1 amu Concentration:1.000 ng/ml Calculated conc:0.481 ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :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:1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.86 Area:1258131 Height:315000 Start Time:1.75 End Time:2.15	
Sample Name:CC4- Fluvastatin sodium Mass(es):415.3/178.0amu Concentration:2.022 ng/ml Calculated conc:1.955 ng/ml Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :200.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:2.09 Area:30281 Height:7090 Area Ratio:0.023700 Start Time:1.97 End Time:2.30	

Sample Name: CC4- : Atorvastatin Mass(es):455.5/165.1 Concentration:1.000 ng/ml Calculated conc:1.995 ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :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:1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.86 Area:1277746 Height:319000 Start Time:1.75 End Time:2.16	
Sample Name: CC5- Fluvastatin sodium Mass(es):415.3/178 amu Concentration:10.111ng/ml Calculated conc:9.933ng/ml Proc.Algorithm: Analyst classsic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time: 2.07 Area:150940 Height:35000 Area Ratio:0.118000 Start Time:1.95 End Time:2.39	
Sample Name: CC5- : Atorvastatin Mass(es):455.5/165.1 amu Concentration:1.000ng/ml Calculated conc:9.933ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :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:1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.85 Area:1281233 Height:322000 Start Time:1.74 End Time:2.13	
Sample Name: CC6- Fluvastatin sodium Mass(es): 415.3/178 amu Concentration:40.443 ng/ml Calculated conc:40. 351 ng/ml Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:2.07 Area:584605 Height:136000 Area Ratio:0.478000 Start Time:1.95 End Time:2.41	

Sample Name: CC6- : Atorvastatin Mass(es):455.5/165.1 amu Concentration:1.000ng/ml Calculated conc: 40. 351 ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:1.85 Area:1221929 Height:306000 Start Time:1.74 End Time:2.13	
Sample Name: CC7- Fluvastatin sodium Mass(es): 415.3/178 amu Concentration: 101.107 ng/ml Calculated conc:101.45 ng/ml Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10 Area Threshold :100 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:2.09 Area:1494406 Height:348000 Area Ratio:1.20000 Start Time:1.95 End Time:2.64	
Sample Name: CC7- : Atorvastatin Mass(es): 455.5/165.1 amu Concentration:1.000ng/ml Calculated conc: 101.45 ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :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:1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.87 Area:1242395 Height:313000 Start Time:1.76 End Time:2.14	
Sample Name: CC8- - Fluvastatin sodium Mass(es): 415.3/178 amu Concentration:202.213 ng/ml Calculated conc:196.099 ng/ml Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :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:2.00 Use Relative RT:False Int. Type: Base To Base Retention Time:2.07 Area:2956131 Height:68600 Area Ratio:2.320000 Start Time:1.95 End Time:2.62	



Supplementry Figure 1: Chromatograms of stock dilutions of standard fluvastatin sodium solution with serum