Original Article

New Method for the *in vivo* Estimation of Fluvastatin and its Application for Pharmacokinetic Studies in Rabbit

Deepthi Sandhala*, Srinivas Lankalapalli

GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh, INDIA.

ABSTRACT

Aim: The study was aimed to conduct a pharmacokinetic evaluation of Fluvastatin in rabbit plasma using a sensitive HPLC method. **Materials and Methods:** The plasma samples were assayed by Waters alliance e-2695 HPLC instrument using symmetry C_{18} column (150x4.6mm, 3.5 μ) under isocratic condition. Here the buffer was 0.1% ortho phosphoric acid. Mobile phase used was acetonitrile and 0.1% ortho phosphoric acid in 50:50 v/v with a flow rate of 1 ml/min. The eluent was monitored at 224 nm for measurement of Fluvastatin. **Results:** The calibration curve was linear over the concentration range of 5-200 ng/ml of Fuvastatin. **Conclusion:** The application denotes all the parameters of system suitability, specificity, linearity and accuracy are in good agreement with USFDA guidelines and applied effectively for the investigation of pharmacokinetic studies in rabbit.

Key words: RP-HPLC, Fluvastatin, Rabbit Plasma, Linearity, Accuracy, Pharmacokinetics.

INTRODUCTION

Fluvastatin is a member of the statin drug class,[1-3] used to treat hypercholesterolemia[4-6] and to prevent cardiovascular disease. Fluvastatin is an antilipemic agent that competitively inhibits hydroxymethylgultaryl-CoenzymeA reeducates. It is fully synthetic HMG-CoA reeducates inhibitor. Some adverse events such as elevation in transaminase levels, headache, dyspepsia, nausea,[7-9] Indigestion,[10-12] Insomnia,[13,14] Myalgia[15] and infrequently rhabdomyolysis.[16,17] Fluvastatin belongs to BCS Class II drug (High permeability and low solubility). Fluvastatin has a short biological life of 1.5-2 hr and molecular formula $C_{24}H_{26}FNO_4$. The Chemical structure of Fluvastatin given in Figure 1. Fluconazole, a potent inhibitor of CYP2C9 increase Fluvastatin levels. In the present study was planned with an objective to conduct *in vivo* pharmacokinetic studies in rabbit. Experimental Fluvastatin sustained release tablet formulation were tried to compare with commercial Fluvastatin extended release tablets. But very few methods of Fluvastatin were reported for *in vivo* estimation in plasma. Hence, for the estimation of Fluvastatin in rabbit plasma it was proposed to develop a new HPLC method.

In Literature[18-27] revealed many HPLC methods and UV methods for the estimation of fluvastatin. But there is no literature report of bio analytical study of fluvastatin in rabbit plasma.

MATERIALS AND METHODS

Materials

Fluvastatin (99.9% pure) was obtained as gift sample from M/s. Aurobindo Pharma Ltd., Hyderabad, India. Commercial tablet of Fluvastatin (Lescol XL) as extended release was bought from local market.

Submission Date: 07-05-2020;
Revision Date: 10-07-2020;
Accepted Date: 23-10-2020

DOI: 10.5530/ijper.54.4.211

Correspondence:
Miss. Deepthi Sandhala
GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam-530045, Andhra Pradesh, INDIA.
Phone no: +91 849-79 71337
Email id: deepthi.sandela@gmail.com

www.ijper.org

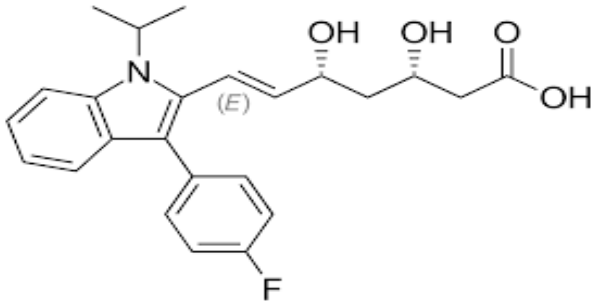


Figure 1: Structural representation of Fluvastatin.

HPLC marked acetonitrile, ortho phosphoric acid were procured from Merck, Mumbai, India. HPLC marked water was obtained from Milli Q (Milli Q system, USA) water purification.

Pharmacokinetic Study

Selection of animals

In vivo pharmacokinetic studies, 6 healthy New Zealand white rabbits (2.0-2.5 kg) were used. All animal experiments approved and performed in GITAM Institute of Pharmacy, Visakhapatnam accord with principles of Institutional Animal Ethics Committee (CPCSEA No: 1287/ac/09/CPCSEA) vide approval IAEC/GIP-1287/LS-F/Approved/1/2019.

In vivo Study Design

The animals are separated into four groups. Before experimentation all animals starved overnight and had water *ad-libitum*. Topical anesthetic procedure was used. Pharmacokinetic evaluation was performed for Fluvastatin solid dispersion tablets (Optimized and Marketed) optimized 20mg and 80mg, marketed 20mg and 80mg. In six different rabbits the dose was administered orally, under fasting condition. Blood samples were collected from rabbit ear vein with volume of 0.2 ml to 0.4 ml at 0.5, 1, 2, 3, 6, 9, 12, 18, 20 and 24 hr. Each sample was separated by centrifugation and stored at -70°C for 20min with 4000rpm.

Analytical method and Instrumentation

In this method, chromatographic condition of HPLC (Waters Alliance e2695) were C_{18} symmetry column (3.5µm, 150mm X 4.6mm) with mobile phase of acetonitrile: 0.1% Ortho phosphoric acid (50:50 v/v) at 1.0 ml/min flow rate under constant temperature of 30°C and detection was done at 224 nm wavelength.

Preparation of Stock Solutions

400 ng/ml of stock solution was made with acetonitrile (5 mg in 25 ml). 40 µg/ml intermediate stock was made with mobile phase from stock. From stock solution, 5, 10, 25, 50, 75, 100, 125, 150 and 200 ng/ml working solutions were made which were used for standard calibration curve. Similarly, 100 ng/ml atorvastatin solution of internal standard (IS) was made.

Sample Preparation

For sample preparation 200 µl of plasma was taken and 500 µl of internal standard and 500 µl of stock solution was added and mixed. To this mixture a 500 µl of acetonitrile was added to precipitate all the proteins and blend within the vortex cycle mixture. This samples further subjected for Centrifuge at 500 rpm for 30 min. Collect the supernatant solution and filter through 0.2 µg filter and the clear solution was placed in HPLC vial for injection into the chromatogram.

Method validation

Specificity

Six rabbit blank plasma samples were collected randomly and they were subjected for protein precipitation. They were chromatographed to identify the endogenous components of plasma which could interfere with either standard drug or internal standard.

System suitability

The system was assured by determining peak retention time, peak area, tailing and plate count of Fluvastatin.

Linearity

Calibration samples of different concentration (5%, 10%, 25%, 50%, 75%, 100%, 125%, 150% and 200%) of Fluvastatin were prepared by appropriate amount and dilution of standard drug into control plasma. The samples were further subjected for protein precipitation and chromatographed.

Accuracy

The accuracy of an analytical method describes mean test results obtained by the nominal value (concentration) of the analyte. Accuracy can be measured by using a minimum of 3 concentrations and 5 determinations per concentration. The deviation of the mean from the nominal value (relative error) serves as the measure of accuracy.

Precision

The precision of an analytical method describes the individual measures of an analyte. Precision can be measured by using a minimum of 3 concentrations and 5 determinations per concentration. Precision is

subdivided into – Within-day precision and – Between-day precision.

Limit of detection and qualification

The Limit of Detection (LOD) is defined as the lowest detectable concentration. Limit of Qualification (LOQ) is defined as lowest quantifiable concentration LOD and LOQ were calculated as 3.3σ/s and 10σ/s respectively. Where 'σ' is standard deviation (SD) of intercept and 's' is slope of calibration curve.

RESULTS AND DISCUSSION

Method Validation

Specificity

No interfering peaks were found in blank plasma sample at the retention time of both Fluvastatin and ISTD. The Chromatogram blank rabbit plasma was shown in Figure 2 and Chromatogram blank plasma spiked with analyte and IS shown in Figure 3.

Standard calibration curve and Linearity

From the calibration curve, it had been clear that the height area ratios were proportional to the concentration. The concentration range of Fluvastatin is 5-200 ng/ml. The calibration curve was appeared linear and coefficient of correlation was found to be 0.999. The regression coefficient value is y=0.0075x+0.0051. The Linearity results of Fluvastatin are given in Table 1 and Figure 4.

Precision

In precision the Quality control sample (MQC), concentration taken was 100 (ng/ml). Precision was determined by %RSD. Intra and inter batch % precision

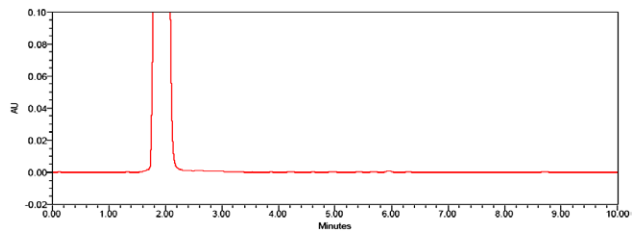


Figure 2: Chromatogram blank rabbit plasma.

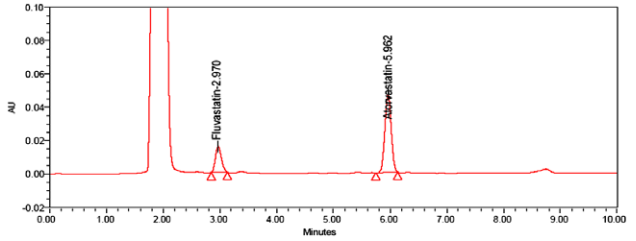


Figure 3: Chromatogram blank plasma spiked with analyte and IS.

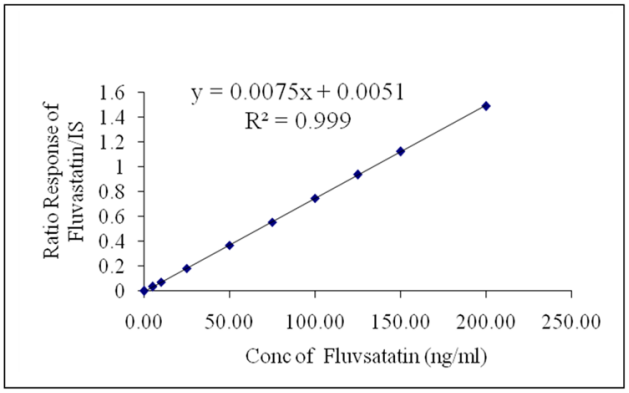


Figure 4: Linearity plot for Fluvastatin

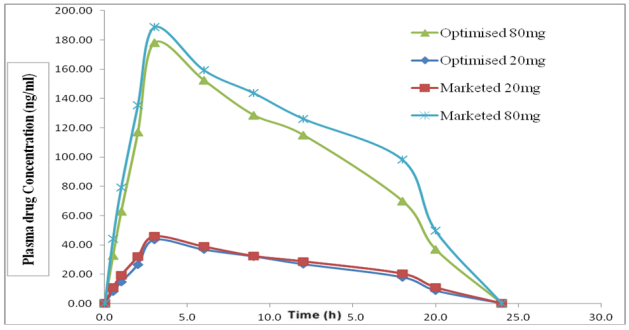


Figure 5: *In vivo* plasma profiles of Fluvastatin in rabbits.

for Fluvastatin was found to be 100.02 and 99.96 and % RSD varies from 0.89 and 1.27. The %CV and precision results were found to be within the suitable limits.

Accuracy

In Accuracy the Quality control sample (LLQC, LQC, HQC), concentration taken was 5, 50, 200 (ng/ml). Accuracy was determined by %RSD. Intra and inter batch % accuracy for Fluvastatin varies from 95.58-100.35 and 96.12-100.82 and %RSD varies form 0.64-1.56 and 0.42-1.27, The %CV and accuracy results were found to be within the suitable limits.

Limit of detection and qualification

The limit of detection (LOD) and Limit of Quantification (LOQ) was estimated from the signal to noise ratio. This limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 3:1 for detection and 10:1 for qualification. The LOD value was found to be 0.5 ng/ml and LOQ values is 5 ng/ml.

Pharmacokinetic Study

Pharmacokinetic studies of Fluvastatin in plasma samples, for calculating the pharmacological parameters with dose of 0.5mg/1.0kg of body mass (equivalent to 20mg of tablet) and 2.0mg/1.0kg of body mass

Table 1: Linearity results of Fluvastatin.				
Linearity	**Fluvastatin concentration (ng/ml)**	**Fluvastatin peak response**	**IS peak response**	**Ratio Response**
1	5	2548	68542	0.037
2	10	5115	68598	0.075
3	25	12578	68745	0.183
4	50	25575	68985	0.371
5	75	37895	68154	0.556
6	100	51154	68240	0.750
7	125	63987	68226	0.938
8	150	76985	68357	1.126
9	200	102874	68974	1.491
Slope			0.075	
Intercept			0.00051	
CC			0.99995	

Table 2: Pharmacokinetic parameters of Fluvastatin.				
Time (hrs)	**Optimized 20mg**	**Marketed 20mg**	**Optimized 80mg**	**Marketed 80mg**
0.0	0.00	0.00	0.00	0.00
0.5	8.24	10.81	32.64	44.09
1.0	14.73	19.23	63.02	79.29
2.0	26.67	32.12	116.93	135.30
3.0	43.63	45.85	177.95	188.69
6.0	36.83	38.88	152.36	159.32
9.0	32.19	32.44	128.63	143.72
12.0	26.90	28.75	115.06	126.17
18.0	17.92	20.35	69.99	98.16
20.0	8.75	10.98	37.05	49.65
24.0	0.00	0.00	0.00	0.00

Table 3: *In vivo* Plasma Profiles of Fluvastatin in Rabbits.				
Pharmacokinetic parameter	**Optimized 20mg**	**Marketed 20mg**	**Optimized 80mg**	**Marketed 80mg**
$AUC_{0\text{-}t\ (ng\ h/ml)}$	556	601	2288	2613
C_{max}	43.6	45.85	178	188.7
$AUC_{0\text{-}\infty(ng\ h/ml)}$	596.5	707.5	2647	3094
$AUC_{t\text{-}\infty(ng\ h/ml)}$	85	106	359	482
$t_{1/2}$	3	3	3	3
T_{max} (h)	3	3	3	3

(equivalent to 80mg of tablet) for both optimized and marketed formulations. Samplings of Fluvastatin in rabbits were taken as follows, optimized 20mg, 80mg and marketed 20mg, 80mg. All the formulations in rabbit are given through oral route. The pharmacokinetic profile of Fluvastatin was depicted in Figure 5 and calculated concentrations were between linearity ranges are given in Table 2.

Pharmacokinetic parameters such as $AUC_{0\text{-}t\ (ng\ h/ml),}$ $AUC_{0\text{-}\infty}$, C_{max}, $AUC_{0\text{-}\infty(ng\ h/ml),}$ $AUC_{t\text{-}\infty(ng\ h/ml)}$ $T_{max,}$ were calculated and the data was shown in Table 3. The $AUC_{0\text{-}t}$ for optimized 20mg and 80mg and was found to be (556 ng h/ml, 2288 ng h/ml), marketed 20mg and 80mg (601 ng h/ml ,2613 ng h/ml). The C_{max} of optimized 20mg and 80mg was found to be (43.6 ng/ml, 178 ng/ml), marketed 20mg and 80mg (45.8 ng/ml and 188.7 ng/ml). The $AUC_{0\text{-}\infty}$ for optimized 20mg

and 80mg was found to be (596.5 ng h/ml, 2647 ng h/ml), marketed 20mg and 80mg (707.5 ng h/ml, 3094 ng h/ml). The $AUC_{t-\infty}$ for optimized 20mg and 80mg was found to be (85 ng h/ml, 359 ng h/ml), marketed 20mg and 80mg (106 ng h/ml, 482 ng h/ml). The T_{max} for optimized 20mg, 80mg and marketed 20mg, 80mg tablets was found to be (3.0 h). The corresponding $t_{1/2}$ for optimized 20mg, 80mg and marketed 20mg, 80mg values were found to 3.0 h. Based on results, it was concluded that the above formulation can help in improving the relative bioavailability.

CONCLUSION

The higher sensitive HPLC method was validated and determined by using Fluvastatin in rabbit plasma. Here the method is fast, rugged, reproducible bio-analytical method. Simple and efficient method was developed and utilized in pharmacokinetic studies to see the investigated analyte in body fluids.

ACKNOWLEDGEMENT

The authors are grateful to GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam for providing necessary facilities and support.

CONFLICT OF INTEREST

Authors declare that there was no conflict of interest.

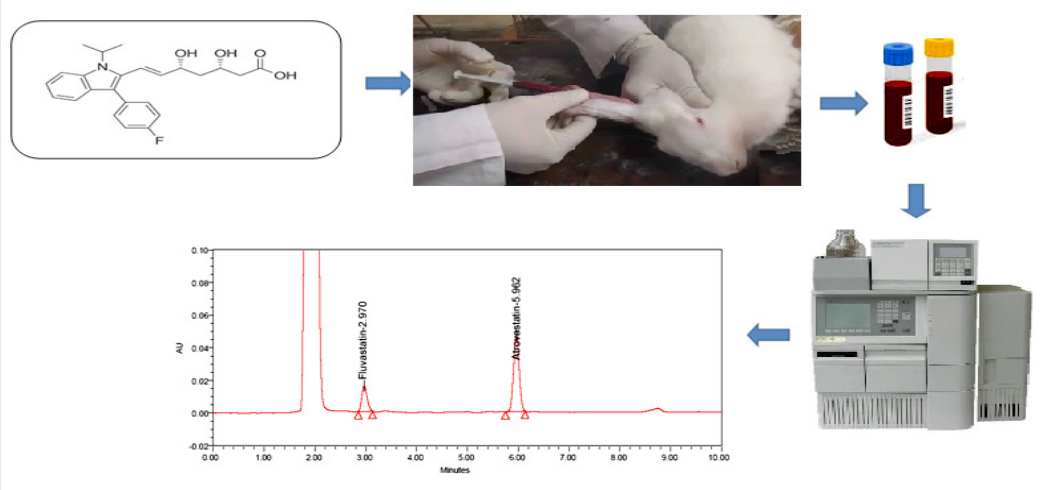
ABBREVIATIONS

RP-HPLC: Reverse Phase High performance liquid chromatography; **NM:** Nano Meter; **USFDA:** United States Food and Drug Administration; **USA:** United States of America; **OPA:** Ortho phosphoric acid; **CV:** Cumulative variation; **AUC:** Area under the ROC curve; **CN:** Propyl-Carbo Nitrile Propyl; **ISTD:** Internal Standard; **%RSD:** Relative Standard Deviation; **%CV:** Cumulative variation **QC:** Quality Control.

REFERENCES

1. Alenghat F, Davis A. Management of Blood Cholesterol. JAMA. 2019;321(8):800.
2. Carmena R, Betteridge D. Diabetogenic Action of Statins: Mechanisms. Current Atherosclerosis Reports. 2019;21(6):23.
3. Lamaida N, Capuano E, Pinto L, Capuano E, Capuano R, Capuano V. The safety of statins in children. Acta Paediatrica. 2013;102(9):857-62.
4. Peters S, Singhateh Y, Mackay D, Huxley R, Woodward M. Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. Atherosclerosis. 2016;248:123-31.
5. Weingartner O, Bohm M, Laufs U. Controversial role of plant sterol esters in the management of hypercholesterolaemia. European Heart Journal. 2008;30(4):404-9.
6. Craeyveld VE, Jacobs F, Gordts CS, DeGeest B. Gene Therapy for Familial Hypercholesterolemia. Current Pharmaceutical Design. 2011;17(24):2575-91.
7. Scorza K, Williams A, Phillips JD, Shaw J. Evaluation of nausea and vomiting. American Family Physician. 2007;76(1):76-84.
8. Singh P, Yoon S, Kuo B. Nausea: A review of pathophysiology and therapeutics. Therapeutic Advances in Gastroenterology. 2015;9(1):98-112.
9. Marx W, Teleni L, McCarthy A, Vitetta L, McKavanagh D, Thomson D, *et al*. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: A systematic literature review. Nutrition Reviews. 2013;71(4):245-54.
10. Duncanson K, Burrows T, Walker M, Talley N. Food and Functional Dyspepsia: A Systematic Review. Gastroenterology. 2017;152(5):S303.
11. Jung H, Talley N. Role of the Duodenum in the Pathogenesis of Functional Dyspepsia: A Paradigm Shift. Journal of Neurogastroenterology and Motility. 2018;24(3):345-54.
12. Futagami S, Itoh T, Sakamoto C. Systematic review with meta-analysis: Post-infectious functional dyspepsia. Alimentary Pharmacology and Therapeutics. 2014;41(2):177-88.
13. Straten AV, Cuijpers P. Self-help therapy for insomnia: A meta-analysis. Sleep Medicine Reviews. 2009;13(1):61-71.
14. Qaseem A, Kansagara D, Forciea M, Cooke M, Denberg T. Management of Chronic Insomnia Disorder in Adults: A Clinical Practice Guideline From the American College of Physicians. Annals of Internal Medicine. 2016;165(2):125.
15. Glueck C, Conrad B. Severe vitamin D deficiency, myopathy and rhabdomyolysis. North American Journal of Medical Sciences. 2013;5(8):494.
16. Chavez L, Leon M, Einav S, Varon J. Beyond muscle destruction: A systematic review of rhabdomyolysis for clinical practice. Critical Care. 2016;20(1):135.
17. Bosch X, Poch E, Grau J. Rhabdomyolysis and Acute Kidney Injury. New England Journal of Medicine. 2009;361(1):62-72.
18. Saminathan J, Sankar ASK, Anandakumar K, Srinivasan S. Validated RP-Hplc Method For Fluvastatin Sodium In Bulk And Its Dosage Form. Journal of Pharmaceutical Sciences and Research. 2009;1(3):90-6.
19. Prakash S, Gopla RP, Sunil KM, Vijay PK, Kiran KV. Analytical method development and Validation for simultaneous estimation of Fluvastatin sodium and Valsartan by RP-HPLC technique. International Journal of Pharmacy and Industrial Research. 2013;03(02):152-7.
20. Fabio PG, Pedro LG, Joao MPA, Anil KS, Erika RMH, Maria IRMS. Development and Validation of Stability Indicating HPLC Methods for Quantitative Determination of Pravastatin, Fluvastatin, Atorvastatin and Rosuvastatin in Pharmaceuticals. Analytical Letters. 2009;42(12):1784-804.
21. Shivani TR, Dilip MG. A Review on Chromatographic and Spectrophotometric method for estimation of Ezetimibe and Fluvastatin. World Journal of Pharmaceutical Research. 2018;7:1014-25.
22. Kalafsky G, Smith HT, Choc MG. High Performance liquid chromatographic method for the determination of Fluvstatin in human plasma. Journal of Chromatography. 1993;614(2):307-13.
23. Ashok HA, Bhanubhai NS, Mahesh GS, Vishnu AS. Development and Validation of Stability indicating RP-HPLC method for estimation of Fluvastatin Sodium in Bulk and Capsule dosage form. Eurasian J Anal Chem. 2017;12(2):87-105.
24. Ashok HA, Umang S, Sagar S, Patel MB. Development and validation of sensitive HPTLC method for quantitative analysis of Fluvastatin sodium in bulk and pharmaceutical dosage form. Journal of Pharmaceutical Research. 2014;6(2):73-8.
25. Tuljapure DS, Gowekar NM, Savita SY. Development and validation of HPTLC method for estimation of Fluvastatin sodium in bulk drug and dosage form. Der Pharmacia Sinica. 2012;3(4):457-61.
26. Prathyusha PH, Anupama B, Jagathi V, Rajesh V. Simultaneous spectrophotometric estimation of Fluvastatin and Fenofibrate in bulk drug and dosage form by using simultaneous equation method. Journal of Pharmaceutical Sciences and Research. 2011;3(2):1042-5.
27. Ashour S, Mahmoud B, Khateeb M. Kinetic spectrophotometric determination of Fluvastatin in pharmaceutical preparations. Int J Biomed Sci. 2010;6(1):19-26.

PICTORIAL ABSTRACT



SUMMARY

The HPLC method describes the analysis of Fluvastatin plasma are very specific and sensitive. The method developed is very simple utilizing liquid-liquid extraction procedure, which makes the method high throughout the analysis. The method shows linearity in concentration range of 5-200 ng/ml. The regression coefficient (R^2) value of the calibration curve is 0.999. The accuracy data concentrations of 5, 50, 100 and 150 ng/ml showed the percentage recovery of 95.58-100.31%. The application denotes all system suitability parameters, specificity, linearity and accuracy are in satisfactory correlation with USFDA directives and effectively applied to investigate pharmacokinetic studies with rabbit plasma.

Cite this article: Sandhala D, Lankalapalli S. New Method for the in vivo Estimation of Fluvastatin and its Application for Pharmacokinetic Studies in Rabbit. Indian J of Pharmaceutical Education and Research. 2020;54(4):1153-8.