INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Paper

Method Development and Validation for The Estimation of Everolimus in Bulk and Pharmaceutical Dosage Form by Using RP-HPLC

Kattu Vennela, M. Ramakrishna Reddy, Chandra sekhar Naik. D*

Nimra College of Pharmacy, Jupudi, Ibrahimpatnam, NTR-521456.

ARTICLE INFO

Published: 24 June 2026
Keywords:
Everolimus, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines
DOI:
10.5281/zenodo.20827487

ABSTRACT

A selective, accurate and precise RP-HPLC method was developed and validated for the estimation of Everolimus in bulk and marketed pharmaceutical dosage forms. The Everolimus was resolved on a Symmetry ODS C18 (4.6mm × 250mm, 5µm) using water: Acetonitrile Buffer used in the ratio of 40:60 % v/v as the mobile phase. The detection wavelength was 235 nm. The retention time obtained for Everolimus were 3.006 min. The linearity ranges were 6-14 with Regression coefficients of 0.9997. The % R.S.D. of precision studies was found to be 0.171. The accuracy of the proposed method was determined by recovery studies and the mean recovery was 99.72%. The limit of detection and the limit of quantitation were found to be 1.4µg/ml and 3.2µg/ml, respectively. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines. Thus the novel proposed method for Everolimus was found to be feasible for the estimation of Everolimus in bulk as well as the pharmaceutical dosage form.

INTRODUCTION

Everolimus is a derivative of Rapamycin (Sirolimus), and works similarly to Rapamycin as an mTOR (mammalian target of Rapamycin) inhibitor. It is currently used as an immunosuppressant to prevent rejection of organ transplants. In a similar fashion to other mTOR inhibitors Everolimus' effect is solely on the mTORC1 protein and not on the mTORC2 protein. Oral Everolimus is absorbed rapidly, and reaches peak concentration after 1.3-1.8 hours. Steady state is reached within 7 days, and steady-state peak and trough concentrations, and area under the concentration-time curve (AUC), are proportional to dosage. Everolimus [1] is an mTOR inhibitor that binds with high affinity to the FK506 binding protein-12 (FKBP-12), thereby forming a drug

***Corresponding Author:** Chandra Sekhar Naik
Address: *Professors, Department of Pharmaceutics, Nimra College of Pharmacy, Jupudi, Ibrahimpatnam, NTR-521456..*
Email : chandu.desavath@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

complex that inhibits the activation of mTOR. This inhibition reduces the activity of effectors downstream, which leads to a blockage in the progression of cells from G1 into S phase, and subsequently inducing cell growth arrest and apoptosis. Everolimus [2] also inhibits the expression of hypoxia-inducible factor, leading to a decrease in the expression of vascular endothelial growth factor. The result of Everolimus [3] inhibition of mTOR is a reduction in cell proliferation, angiogenesis, and glucose uptake. The IUPAC Name of Everolimus is (1R, 9S, 12S, 15R, 16E, 18R, 19R, 21R, 23S, 24E, 26E, 28E, 30S, 32S, 35R)-1, 18-dihydroxy-12-[(2R)-1-[(1S, 3R, 4R)-4-(2-hydroxy ethoxy)-3-methoxy cyclo hexyl] propan-2-yl]-19, 30-dimethoxy-15, 17, 21, 23, 29, 35-hexa methyl-11, 36-dioxa-4-azatri cyclo [30.3.1.04, 9] hexatriaconta-16, 24, 26, 28-tetraene-2, 3, 10, 14, 20-pentone. The Chemical Structure of Everolimus as shown in fig-1.

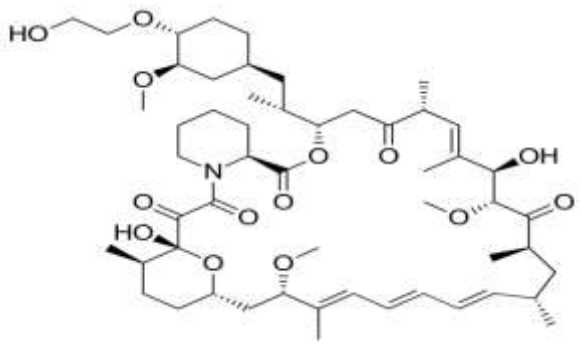


Fig 1: Chemical Structure of Everolimus

MATERIALS AND METHODS:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Everolimus solutions.

METHOD DEVELOPMENT:

Preparation of standard solution

Accurately weigh and transfer 10 mg of Everolimus working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Everolimus stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase4 tried was water: Acetonitrile Buffer with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 40:60% v/v.

Standard Preparation: (Everolimus 100µg/ml)

Accurately Weighed and transferred 10mg Everolimus working Standard into a 10ml clean dry volumetric flask, add 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution, 1 ml was pipeted out in to a 10ml Volumetric flask and then make up to the final volume with diluents**.**

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent. **Buffer:** 0.77gms of

ammonium acetate was dissolved in 1000ml HPLC grade water and sonicated for 30mins.

Optimization of Column

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Everolimus working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1ml of the above Everolimus stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Everolimus working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1ml of the above Everolimus stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Linearity

Accurately weigh and transfer 10 mg of Everolimus working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Precision

Repeatability

Preparation of Everolimus Product Solution for Precision

Accurately weigh and transfer 10 mg of Everolimus working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1ml of the above Everolimus stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Accuracy

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Everolimus working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.05ml of the above Everolimus stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

RESULTS AND DISCUSSIONS

Optimized Chromatographic Conditions

Column : Std ODS 250mm x 4.6 mm, 5µ.
Mobile phase : water: Acetonitrile (40:60)
Flow rate : 1.0 ml/min
Detector : PDA 268nm
Temperature : 30^0C
Injection Volume : 10µL

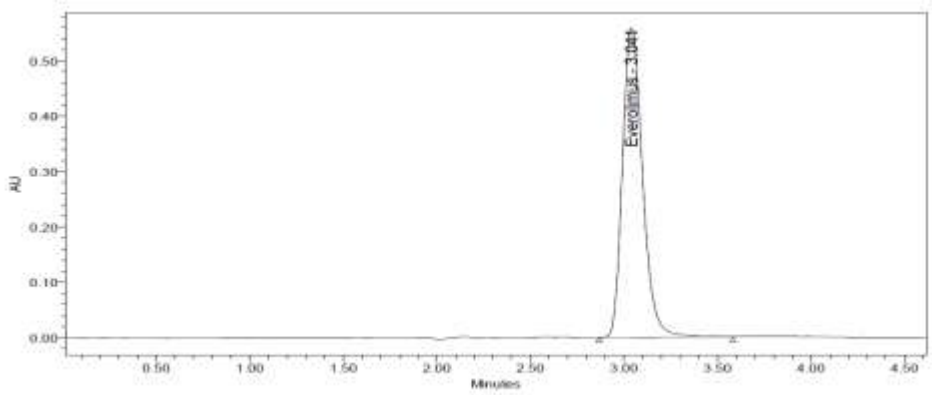


Fig 2: optimized chromatogram

SYSTEM SUITABILITY

A Standard solution of Everolimus working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table 2.

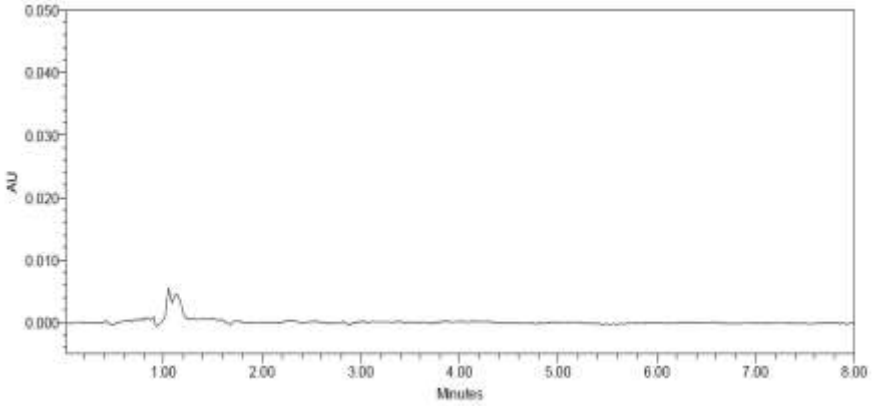


Fig no 3: blank Chromatogram

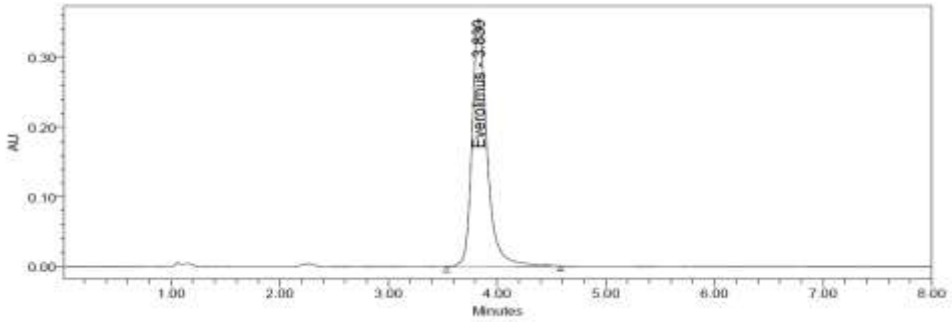


Fig no 4: System suitability Chromatogram

Precision:

Repeatability: Six working sample solutions of 100ppm are injected and the % Amount found was calculated and %RSD was found to be 0.83 and chromatogram was shown in fig 3 & 4.

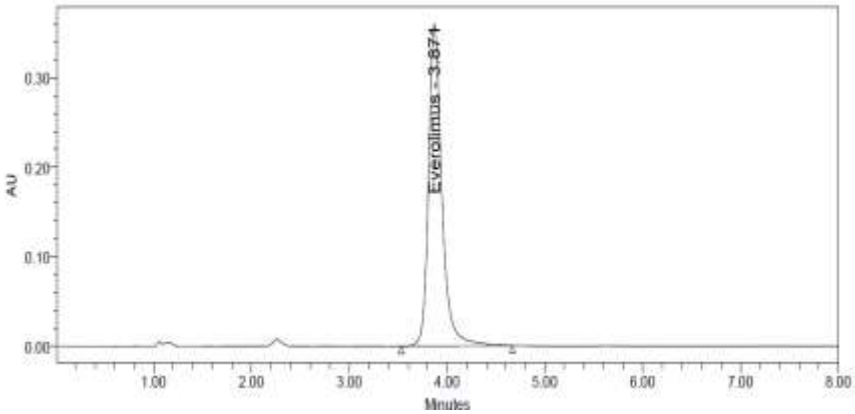


Fig no 5: Repeatability Chromatogram

Intermediate precision: Six working sample solutions of 100ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.27 and chromatogram was shown in fig 5.

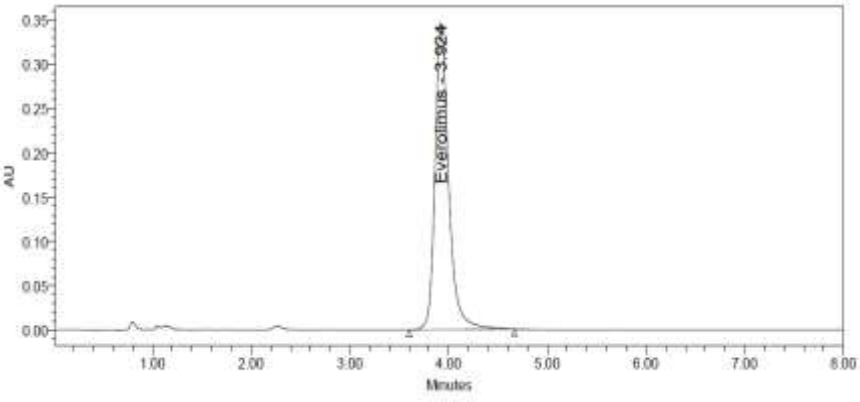


fig no 6: Intermediate precision Chromatogram

LINEARITY:

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 25ppm to 150ppm of Everolimus . Plot a graph to concentration versus peak area. Slope obtained was 45698 Y-Intercept was 32527 and

Correlation Co-efficient was found to be 0.9997 and Linearity plot was shown in Fig 6.

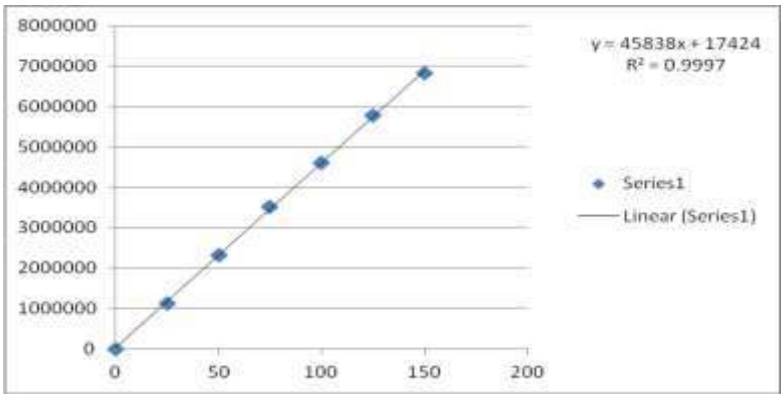
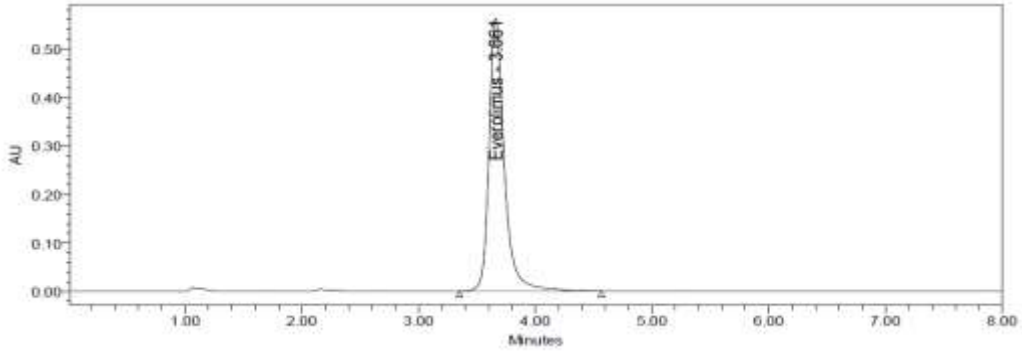
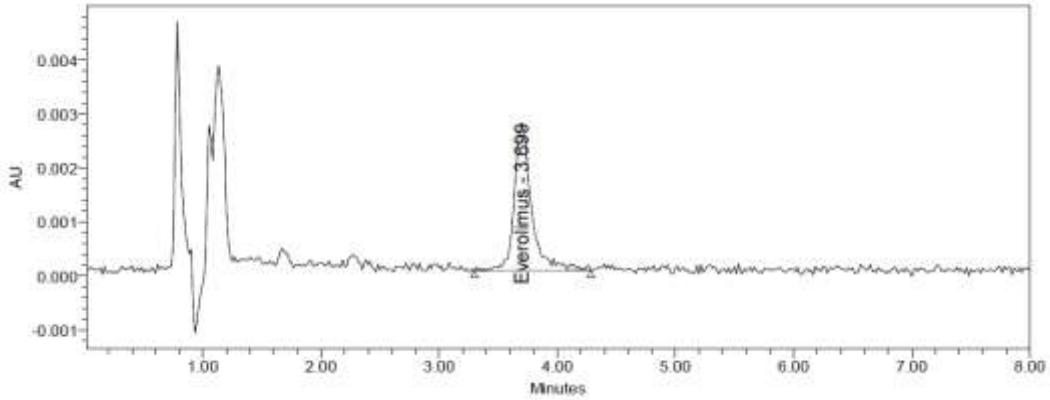


Fig no:7 Linearity Plot

Accuracy: Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recover was calculated as 100.24%. And %RSD was found to be 0.69 and chromatograms were shown in fig no 7.



LOD: Ditection limit of the Everolimus in this method was found to be 0.061µg/ml.



LOQ: Quantification limit of the Everolimus in this method was found to be 0.149µg/ml.

Robustness: Small Deliberate change in the method are made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated.

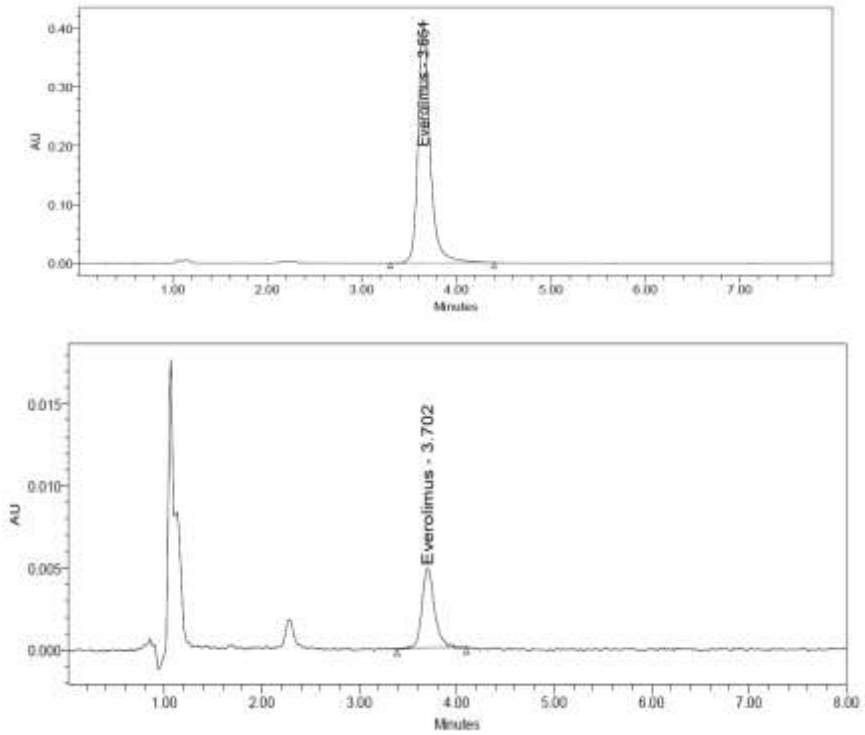


Fig no: 7 LOQ Chromatogram of Everolimus

CONCLUSION

A robust, accurate, precise, and sensitive RP-HPLC method was successfully developed and validated for the quantitative determination of Everolimus in pharmaceutical dosage forms. Chromatographic separation was achieved using a Std ODS (250 mm × 4.6 mm) column with a mobile phase consisting of buffer and acetonitrile (40:60 v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 268 nm, with the column maintained at 30°C. The developed method exhibited satisfactory system suitability and complied with all validation requirements. Excellent linearity was observed over the concentration range of 25–150%, with a correlation coefficient (R^2) of 0.9997. The method demonstrated high precision, with %RSD values of 0.83% for repeatability and 0.27% for intermediate precision. The low values obtained for LOD (0.061 µg/mL) and LOQ (0.149 µg/mL) indicated the high sensitivity of the method for detecting and quantifying Everolimus. Application of the validated method to the analysis of a marketed formulation showed an assay value of 100.31%, confirming its accuracy and suitability for routine pharmaceutical analysis. Therefore, the proposed RP-HPLC method can be effectively employed for quality control, assay determination, and routine analytical evaluation of Everolimus in bulk drug substances and pharmaceutical formulations.

REFERENCES

1. R Snyder, J Kirkland, L Glajch. Practical HPLC Method Development, john Wiley and sons International publication, II Edn.; c2011.
2. S Ashutoshkar. Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers; c2005:452-474.
3. H Beckett, JB Stenlake. Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors New Delhi, 1-9, 157-167.
4. HH Williard, LL Merit, FA Dean, FA Settle. d430-440, 495-504, 529-545.

5. BK Sharma. Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut, 286-300.
6. Instant notes on analytical chemistry by D. Kealey and P.J. Haines, © BIOS Scientific Publishers Limited, UK; c2002:6-7.
7. Gurdeep R Chatwal, Sham K Anand. Instrumental methods of Chemical Analysis, 5th edition, Himalaya Publishing House (Mumbai); c2005:2.566.
8. ME Swartz. Journal of liquid chromatography. 2005;28(7/8):1253-1263.
9. Journal of Chromatography. B, Analytical Technologies in the Biomedical and life Sciences. 2008 March 1. Published on. 2008;863(2):258-2650.
10. International Conference on Harmonization Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1); c2005.
11. International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A; c1994.
12. JM Green. A practical guide to analytical method validation, anal. Chem. News & features; c1996:305a-309a.
13. PA Winslow, rf Meyer. Defining a master plan for the validation of analytical methods, J Validation technology; c1997:361-367.
14. Aoac peer-verified methods program, manual on policies and procedures, Arlington, Va., USA; 1998.
15. R Patil. J of Chromatographia. c2008;67:575.
16. Baht, Leena. J of Liq. Chrom. c2007;30:309.
17. HH Williard, LL Merit, FA Dean, FA Settle. Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi; 2002.
18. GN Menon, LB White. Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).
19. Food, Drug Administration (FDA), Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices). 2000;65 (169):52776-52777.
20. Vibha G, *et al*. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(4):22-23.
21. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc.; c2006:88-92.
22. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva; 1996:11. (CPMP/ICH/281/95).
23. Development and validation of HPLC method - A Review, Vibha Gupta *et al*, International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(4):17-25.

HOW TO CITE: Kattu Vennela, M. Ramakrishna Reddy, Chandra sekhar Naik. D, Method Development and Validation for The Estimation of Everolimus in Bulk and Pharmaceutical Dosage Form by Using RP-HPLC, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 6, 6055-6062, https://doi.org/10.5281/zenodo.20827487