WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 14, Issue 24, 560-569. Review Article ISSN 2277–7105

A REVIEW ON DEVELOPMENT AND VALIDATION OF METHOD FOR DETERMINATION OF SOME RECENT MARKETED FORMULATION CONTAINING ROSUVASTATIN AND EZETIMIBE

Mamta Devidas Dhande*, Dr. Sachin C. Kale, Dr. Kailash R. Biyani

Anuradha College of Pharmacy, Anuradha Nagar, Sakegaon Road. Chikhli, Dist-Buldana, Maharastra (India) 443201.

Article Received on 15 Nov. 2025,
Article Revised on 05 Dec. 2025,
Article Published on 16 Dec. 2025,

https://doi.org/10.5281/zenodo.17949828

***Corresponding Author**

Mamta Devidas Dhande

Anuradha College of Pharmacy, Anuradha Nagar, Sakegaon Road. Chikhli, Dist-Buldana, Maharastra (India) 443201.

How to cite this Article: Mamta Devidas Dhande*, Dr. Sachin C. Kale, Dr. Kailash R. Biyani (2025). A Review On Development And Validation Of Method For Determination Of Some Recent Marketed Formulation Containing Rosuvastatin And Ezetimibe. World Journal of Pharmaceutical Research, 14(24), 560–569.

ABSTRACT

Rosuvastatin (RST) is chemically designated as (3R, 5S, 6E) - 7 - [4 - (4 - fluorophenyl) - 2 - (N - methylmethanesulfonamido) - 6 - (propan - 2 - yl) pyrimidin - 5 - yl] - 3, 5 - dihydroxyhept - 6 - enoic acid. It is a member of the drug class of statins. It reduces levels of low-density lipoprotein, apolipoprotein B and triglycerides in the blood, while increasing levels of high-density lipoprotein in the management of hyper lipidaemias. Ezetimibe (EZT) chemically designated as (3R, 4S) - 1 - (4 - fluorophenyl) - 3 - [(3S) - 3 - (4 - fluorophenyl) - 3 - hydroxypropyl] - 4 - (4 - hydroxyphenyl) azetidin - 2 – one. It is a selective cholesterol absorption inhibitor, used for the treatment of hyperlipidemia, which potentially inhibits the absorption of biliary and dietary cholesterol. Future validations will focus on demonstrating method reliability across various laboratories, ensuring resilience to minor fluctuations in testing conditions, thus enhancing reproducibility and compliance with International Conference on Harmonisation (ICH) guidelines. The integration of automation in sample preparation and analysis is expected to improve operational efficiency, with Process Analytical Technology (PAT) allowing for real-time monitoring during manufacturing processes. This approach will yield high-quality data and necessitate sophisticated validation protocols to maintain regulatory compliance. An analytical method using reversed-phase high-performance liquid chromatography (RP-HPLC) was developed and validated for the simultaneous determination

of rosuvastatin and ezetimibe in commercial tablet formulations. This method, validated according to ICH guidelines, is described as simple, sensitive, accurate, precise, linear, robust, and specific, making it suitable for routine quality control and stability studies.

KEYWORDS: RP-HPLC, Rosuvastatin, Ezetimibe, Isocratic, ICH guidelines.

1. INTRODUCTION

Rosuvastatin is a statin medication used as a lipid-lowering agent. The FDA-approved indications are homozygous familial hypercholesterolemia, hyperlipidemia, mixed dyslipidemia, primary dysbetalipoproteinemia, hypertriglyceridemia, and prevention of cardiovascular disease (Figure 1). The non-FDA-approved uses are in non-cardioembolic stroke, secondary prevention in transient ischemic attack (TIA), and perioperative therapy for cardiac risk reduction in noncardiac surgeries. This activity outlines the indications, mechanism of action, methods of administration, important adverse effects, contraindications, and monitoring, of rosuvastatin, so providers can direct patient therapy in treating hyperlipidemia as part of the interprofessional team.[1-3]

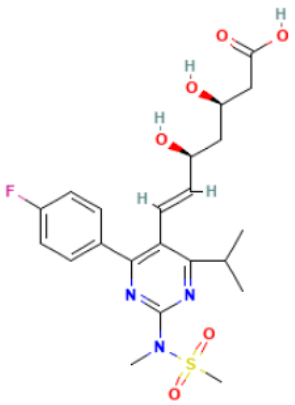

Figure 1: Rosuvastatin.

Dosage for the oral tablet is the same for the generic and trade name: 5 mg, 10 mg, 20 mg, 40 mg. Dosing is individualized based on the low-density lipoprotein (LDL) levels at baseline and the goal of therapy. Adjustments should be made at a regular interval of four weeks or more depending on the underlying pathology of the disease and whether the treatment is for primary versus secondary prevention. Due to the risk of significant drug-drug interactions, the adjustment in dosing should be part of medication reconciliation with the current literature and a pharmacist.[4-6]

Ezetimibe is a beta-lactam that is azetidin-2-one which is substituted at 1, 3, and 4 by p-fluorophenyl, 3-(p-fluorophenyl)-3-hydroxypropyl, and 4-hydroxyphenyl groups, respectively (the 3R,3'S,4S enantiomer) (Figure 2). It has a role as an anticholesteremic drug, an antilipemic drug and an antimetabolite. It is a member of azetidines, an organofluorine compound and a beta-lactam.[7-8] Unlike other classes of cholesterol-reducing compounds including statins and bile acid sequestrants, ezetimibe has a distinct mechanism of action involving the sterol transporter Niemann-Pick C1-Like 1 (NPC1L1), and is unique in that it does not affect the absorption of fat-soluble nutrients such as fat-soluble vitamins, triglycerides, or bile acids. In genetically NPC1L1-deficient mice, a 70% reduction in intestinal cholesterol absorption was seen, and these mice were insensitive to ezetimibe treatment - it was determined based on these findings that NPC1L1 plays an essential role in promoting intestinal cholesterol uptake via an ezetimibe-sensitive pathway. By interfering with the intestinal uptake of cholesterol and phytosterols, ezetimibe reduces the delivery of intestinal cholesterol to the liver.[9-11]

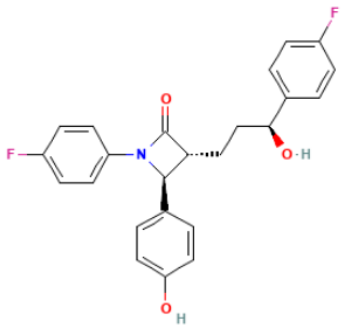

Figure 2: Ezetimibe.

2. Review on Development and Validation of Method for Determination of Some Recent Marketed Formulation Containing Rosuvastatin and Ezetimibe

Patel BD et al., 2024 developed RP-HPLC method was optimized for synchronized analysis of Efonidipine Hydrochloride Ethanolate (EFE) and Chlorthalidone (CHL) in tablets. The separation was performed using an Inertsil ODS C18 column and PDA detector. The optimum conditions were selected based on KH2PO4 concentration and flow rate. The optimized HPLC condition met ICH acceptance criteria, and the linear calibration curve was within the range of 6.25-18.75 and 20-60 µg/ml. The validated method can be used for routine tablet analysis.[12]

Solanki KH et al., 2024 developed a high-performance thin layer chromatographic method has been developed to determine efonidipine hydrochloride (EFD) and telmisartan (TEL) in tablet dosage form for hypertension treatment. The method uses aluminum plates coated with silica gel 60 F254 as a stationary phase and n-butanol: toluene: acetic acid as a mobile phase for separation. Compact spots of TEL and EFD were obtained, and densitometric detection was carried out at 299 nm in UV. The method was validated according to ICH guidelines and successfully applied for tablet formulation estimation. The method's greenness score was 0.69, making it greener.[13]

Dharuman N et al., 2024 developed a green RP-HPLC approach for simultaneous estimation of Cilnidipine (CIL) and Rosuvastatin (MET) in pharmaceutical formulations. The approach uses an integrated experimental strategy, combining design of experiment (DOE) and green analytical chemistry (GAC). The separation process uses an Inertsil ODS 3 column, gradient mobile phase, and phosphate buffer. The linearity is established over a range of 7-13 µg/ml for CIL and 35-65 µg/ml for MET. The technique has been validated according to ICH Q14 and its green nature was examined by GAPI, AES, and AGREE.[14]

Vilela de Oliveira C et al., 2024 developed a new green ultra high performance liquid chromatography (UHPLC) method was developed for determining Rosuvastatin (MET) and hydrochlorothiazide (HCT) in a binary tablet. The method used a Zorbax® SB-C18 column with isocratic elution and separated the analytes and HCT degradation product. The method was found to be accurate, precise, linear, and selective, making it suitable for pharmaceutical quality control and eco-friendly compared to other methods.[15]

GAJRE DZ et al., 2024 developed a simple, sensitive and accurate Development and validation of analytical method for estimation of Efonidipine hydrochloride, Ethanolate and Chlorthalidone in synthetic. A reversed-phase high performance liquid chromatography method is developed and validated for the determination of three drugs. With the help of RP-HPLC it gives us to good resolution and better separation of three drugs. The separation was conducted by using Cybersil C18 column (250mm x 4.6mm x 5?m) with mobile phase consisting Potassium dihydrogen phosphate: Methanol: Acetonitrile (30:30:40 v/v/v) (pH :3). The mobile phase was delivered at flow rate of 1.0 ml/min. m. During the forced degradation studies, Efonidipine showed maximum degradation (10 %) under oxidative stress followed by 8 % photodegradation. The drug showed lower degradation under acid, base and thermal stress conditions, to the extent of 4 %, 3 % and 6 % respectively. It was also observed that the

retention time of the degradant under photolytic and oxidative degradation were the same probably due to the formation of the same product. Therefore, proposed method can be successfully used for routine analysis of Efonidipine hydrochloride, Ethanolate and Chlorthalidone in bulk as well as synthetic mixture.[16]

Bharati S et al. 2023 developed a method involved methanolic extraction, chromatographic separation, and UV detection. The retention time of EFE was 5.2 minutes. The method was validated with parameters within the International Council for Harmonization guidelines. It was successfully used to evaluate pharmacokinetic properties in Wistar albino rats after oral administration of 10 mg/kg of EFE. This simple method enables quick and affordable detection of EFE from biological material.[17]

Kumar TH et al. 2023 developed a new RP-HPLC method has been for estimating Rosuvastatin and hydrochlorothiazide in pharmaceutical formulations. The method uses an Enable C 18G column with a mobile phase of acetonitrile and trifluoroacetic acid in water. UV detection was performed at 222 nm. The method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was observed over a concentration range of 2-40 µg/ml for Rosuvastatin and 0.5-80 µg/ml for hydrochlorothiazide. The method was found to be accurate and precise under various degradation conditions, including acidic, alkaline, oxidative, thermal, and photolysis.[18]

Darji H et al. 2023 focuses on the use of HPLC and Absorbance Correction UV methods for determining Azelnidipine and Rosuvastatin in synthetic mixtures. The HPLC chromatography was performed using a gradient technique on a reversed-phase C18 column with mobile phase based and optimized depending on the polarity of the molecules. The retention time was found to be 6.367 and 2.308 minutes for Azelnidipine and 50.0–150.0 µg/mL, respectively. The method achieved chromatographic separation over linearity 10.0 - 30.0 µg/mL and 50.0–150.0 µg/mL with r2 0.9995 and 0.9993, respectively. The system suitability test parameters were found to be 7042.56 (AZL) and 2231.74 (MET), and tailing factor was 1.47 (AZL) and 1.52 (MET).[19]

Sudha T et al. 2023 focuses on optimizing the LCMS method for estimating the concentration of three antihypertensive drugs, Telmisartan, Chlorthalidone, and Rosuvastatin in commercial pharmaceutical preparations. Twenty experiments were conducted, analyzing retention time, resolution, and peak area. The results were fitted into a second-order

polynomial to predict optimal conditions for effective compound separation. The optimum conditions were acetonitrile and potassium dihydrogen orthophosphate buffer, with a spray voltage of 2.740 V. The m/z range for Telmisartan, Chlorthalidone, and Rosuvastatin was found to be 481.05 → 113.25, 325.05, 585.15 → 229.10, respectively. The optimized assay condition was validated according to International Conference on Harmonization guidelines for specificity, linearity, accuracy, and precision.[20]

Solanki D et al. 2022 developed a method for simultaneous estimation of these drugs. Method I uses Vierodt's Method, which measures absorption at 251 and 227 nm, while Method II uses a first-order derivative. Linearity was observed in concentration ranges of 6.4-38.4 µg.mL-1 for Efonidipine hydrochloride ethanolate and 2-12 µg.mL-1 for Chlorthalidone using methanol as a solvent. The accuracy of the methods was found to be within the range of 98-102% for both drugs. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory.[21]

Dudhrejiya A et al. 2022 developed a method has been developed and validated for estimating Efonidipine Hydrochloride Ethanolate and Telmisartan in synthetic mixtures. The method uses first-order derivative overlay spectra for quantification, determining both drugs at their zero crossing point working wavelength. The method follows the International Conference on Harmonization (ICH) (Q2R1) guideline, and the Beer-Lambert's law is obeyed in the concentration range of 2-18 µg/ml and 4-36 µg/ml for Efonidipine Hydrochloride Ethanolate and Telmisartan, respectively. The percentage recovery ranges from 98-101 % for Efonidipine Hydrochloride Ethanolate and 98.46-99.77% for Telmisartan.[22]

Thakker N et al. 2022 developed a method was carried out using a Shimadzu Shimpack-C18 GIST AQ column and mobile phase A and B were formic acid and acetonitrile respectively. The analysis was completed within 4 minutes at a flow rate of 0.3mL/min. The method was validated in terms of linearity, accuracy, precision, lower limit of quantitation, method sensitivity, and various solution stability parameters, meeting ICH, EMA, and FDA guidelines.[23]

3. Future Scope

The development and validation of analytical methods for rosuvastatin and ezetimibe combinations are poised for significant advancements, focusing on modern techniques and innovative applications. Key areas of future development include: The pursuit of expedited

analytical methods embraces Ultra-Performance Liquid Chromatography (UPLC), which offers superior resolution and shorter runtimes compared to conventional High-Performance Liquid Chromatography (HPLC). This is critical for translating laboratory methods into practical applications capable of handling higher sample volumes. Research emphasizes the role of nano-drug delivery systems in improving the bioavailability and dissolution rates of rosuvastatin and ezetimibe. Tailored extraction and quantification methodologies for these drugs from nanoparticle formulations will be crucial for quality assurance in pharmaceutical production. Transitioning from UV detection to LC-MS/MS enhances sensitivity and specificity, particularly for analyzing drug concentrations within complex biological matrices such as human plasma, serum, and urine. This shift is pivotal for pharmacokinetic studies and clinical trial applications. A paradigm shift towards more sustainable analytical practices is observed, promoting procedures that minimize the use of hazardous solvents. This aspect is essential to meet current environmental standards and regulatory requirements in drug analysis.

Future validations will focus on demonstrating method reliability across various laboratories, ensuring resilience to minor fluctuations in testing conditions, thus enhancing reproducibility and compliance with International Conference on Harmonisation (ICH) guidelines. Developing and validating methods that distinguish and quantify active pharmaceutical ingredients from their degradation components will be vital for ongoing stability studies, ensuring product integrity over extended periods and under accelerated conditions. Existing bioequivalence studies generally target healthy subjects in fasting states, necessitating future investigations that include diverse demographics such as elderly patients or those with comorbidities, alongside the variable influence of food consumption on drug action. The integration of automation in sample preparation and analysis is expected to improve operational efficiency, with Process Analytical Technology (PAT) allowing for real-time monitoring during manufacturing processes. This approach will yield high-quality data and necessitate sophisticated validation protocols to maintain regulatory compliance.

4. CONCLUSION

An analytical method using reversed-phase high-performance liquid chromatography (RP-HPLC) was developed and validated for the simultaneous determination of rosuvastatin and ezetimibe in commercial tablet formulations. This method, validated according to ICH

guidelines, is described as simple, sensitive, accurate, precise, linear, robust, and specific, making it suitable for routine quality control and stability studies.

5. Conflict of Interest

None.

6. REFERENCES

1. Sayehmiri K, Shohani M, Qavam S, Tavan H. Comparing the effectiveness of Rosuvastatin and Atorvastatin on changes in LDL, TG and HDL: A systematic review and meta-analysis. Life Sciences, 2025 Mar 24: 123576.
2. Singh H, Kaur S, Kaushal P, Sharma J, Singla M. Risk of new onset diabetes mellitus with pitavastatin as compared to atorvastatin and rosuvastatin: a systematic review and meta-analysis. Expert Review of Clinical Pharmacology, 2024 Dec 1; 17(12): 1173-81.
3. Kang Y, Park JM, Lee SH. Moderate-intensity rosuvastatin/ezetimibe combination versus quadruple-dose rosuvastatin monotherapy: a meta-analysis and systemic review. Yonsei Medical Journal, 2023 Dec 13; 65(1): 19.
4. Mirghani HO, Asiri KM, Hussain SH, Alqahtani MA, Albaridi RF, Hader KY, Alanzi MF, Aljohani RJ, Alqarni RS, Alturki NA, Albalawi RK. Atorvastatin vs Rosuvastatin in the Prevention of Cardiovascular Events: A Systematic Review. Saudi Medical Horizons Journal, 2024 Oct 14; 4(3): 191-202.
5. Mostaza JM, Escobar C. Rosuvastatin-based lipid-lowering therapy for the control of LDL cholesterol in patients at high vascular risk. Journal of Clinical Medicine, 2024 Mar 25; 13(7): 1894.
6. Alrajeh K, Roman YM. The frequency of rs2231142 in ABCG2 among Asian subgroups: implications for personalized rosuvastatin dosing. Pharmacogenomics, 2023 Jan 1; 24(1): 15-26.
7. Huang J, Li H, Wang X, Liang X, Zhao T, Hu J, Bai H, Ge J, Sun S, He J. Impacts of ezetimibe on risks of various types of cancers: a meta-analysis and systematic review. European Journal of Cancer Prevention, 2023 Jan 1; 32(1): 89-97.
8. Manolis AA, Manolis TA, Mikhailidis DP, Manolis AS. Are we using Ezetimibe as much as we should?. Biomarker Insights, 2024 May; 19: 11772719241257410.
9. Omidi F, Rahmannia M, Shahidi Bonjar AH, Mohammadsharifi P, Nasiri MJ, Sarmastzadeh T. Ezetimibe and atherosclerotic cardiovascular disease: a systematic

review and meta-analysis. Frontiers in Cardiovascular Medicine, 2023 Nov 24; 10: 1269172.

10. Boskabadi AR, Khodabandelu S, Rahimi Y, Motamedi A, Asili P, Ghasempour A, Keshavarzian A, Noori S, Rahmanian M. Efficacy and Safety of Ezetimibe for Non-Alcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Current Reviews in Clinical and Experimental Pharmacology, 2025.
11. Priani SE, Chaerunisaa AY, Wilar G, Sopyan I. Formulation strategies for ezetimibe and its combinations: advancing biopharmaceutical and therapeutic potential. Drug Design, Development and Therapy, 2025 Dec 31: 8555-80.
12. Patel BD, Vekaria HJ. Central Composite Design Expert-Supported RP-HPLC Optimization and Quantitative Evaluation of Efonidipine Hydrochloride Ethanolate & Chlorthalidone in Tablet. Journal of Chromatographic Science, 2024 Jul; 62(6): 585-92.
13. Solanki KH, Solanki DP, Shah DA, Chhalotiya UK, Kachhiya HM, Tandel JN, Parmar MS. HPTLC–densitometric method of analysis for estimation of efonidipine hydrochloride and telmisartan used in treatment of hypertension. Journal of Liquid Chromatography & Related Technologies, 2024 Aug 31: 1-0.
14. Dharuman N, Santhana Lakshmi K, Krishnan M. Design of experiment driven ecofriendly RP-HPLC for simultaneous determination of Cilnidipine and Rosuvastatin. Analytical Chemistry Letters, 2024 May 24: 1-20.
15. Vilela de Oliveira C, Peron A, Rashid A, Gavilano Fajardo FA, Rebello Lourenço F, Maggi Tavares MF, Aurora Prado MS. Validation, measurement uncertainty estimation and evaluation of UHPLC greenness for simultaneous determination of Rosuvastatin and hydrochlorothiazide in binary tablet. Journal of Taibah University for Science, 2024 Dec 31; 18(1): 2339010.
16. RITIKA GAJRE DZ, Jain V, GAJRE R, Dedania Z. STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF EFONIDIPINE HYDROCHLORIDE ETHANOLATE AND CHLORTHALIDONE. IJRAR-International Journal of Research and Analytical Reviews (IJRAR), 2023 Apr; 10(2): 606-48.
17. Bharati S, Gaikwad V, Chellampillai B. Bioanalytical method development and Validation for determination of efonidipine hydrochloride ethanolate in rat plasma by high-performance liquid chromatography: applications for pharmacokinetic study. Analytical Chemistry Letters, 2023 Jul 4; 13(4): 394-402.

18. Kumar TH, Ravindar B, Rasheed SH, Gajji N. Stability-indicating Rp-HPLC method for the estimation of Rosuvastatin and Hydrochlorothiazide in tablet dosage form. International Journal of Pharmaceutical Technology Letters, 2023 Jun 13; 1(1): 17-26.
19. Darji H, Dedania Z. Simultaneous estimation of Azelnidipine and Rosuvastatin with greenness assessment using HPLC and UV-spectrophotometric methods. Green Analytical Chemistry, 2023 Dec 1; 7: 100079.
20. Sudha T, Tamizhselvi S, Dinesh KS, Prabhakar V, Muthuraj M, Vivekanandan N. METHOD DEVELOPMENT AND VALIDATION OF TELMISARTAN, CHLORTHALIDONE AND ROSUVASTATIN USING RP-LC/MS EXPERIMENTAL DESIGN METHOD (CCD) IN BULK AND TABLET DOSAGE FORM. Eur. Chem. Bull, 2023; 12(Special Issue 5): 01–07.
21. Solanki D, Patel D, Meshram D. Development and validation of UV Spectrophotometric method for simultaneous estimation of Efonidipine hydrochloride ethanolate and Chlorthalidone in their synthetic mixture. Drug Analytical Research, 2022 Jul 28; 6(1): 27-34.
22. Dudhrejiya A, Patel A, Chavda J, Gol D, Koli P. Spectrophotometric simultaneous determination of efonidipine hydrochloride ethanolate and telmisartan in synthetic mixture by first order derivative method. J Med Pharam Allied Sci, 2022; 11(2): 4547-51.
23. Thakker N, Shinde G, Dharamsi A, Choudhari V, Pawar S. Development and Validation of Bioanalytical Method for Simultaneous Estimation of Olmesartan medoximil and Rosuvastatin by UHPLC-MS/MS in human plasma. Research Journal of Pharmacy and Technology, 2022; 15(7): 2909-16.