RESEARCH PAPER

Development and validation of RP-HPLC analytical method for simultaneous estimation of Teneligliptin and Rosuvastatin in dosage form

Abhilasha Kumari[1*], Sushila Kaura[1]
[1]Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan
***Corresponding Author**
Abhilasha Kumari
Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan
Email: abhilashadubey2408@gmail.com

ABSTRACT
A new RP-HPLC method was developed for the estimation of teneligliptin and rosuvastatin in tablets and it was validated as per ICH guidelines. The chromatogram was found to be satisfactory on symmetry C-18 (4.6×250mm, 5µ Shim-Pack Solar column) using formic acid (pH 2.65) and acetonitrile in the ratio of 50:50 v/v at a flow rate of 1.0 ml/min. The retention time of teneligliptin and rosuvastatin were found to be 2.027 min and 7.787 min respectively at 265 nm. The system suitability parameters proved that the proposed method is suitable for simultaneous estimation of teneligliptin and rosuvastatin. Tailing factor for the peak was found to be 1.277 and 0.956 for teneligliptin and rosuvastatin respectively and the theoretical plates for separation were found to be 9397 and 153741 respectively for teneligliptin and rosuvastatin. The method was found to be linear in the range of 6-30 µg/ml for both the drugs. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD was found to be 2.257 µg/ml for teneligliptin and 2.338 µg/ml for rosuvastatin while the LOQ was found to be 6.840 µg/ml for teneligliptin and 7.086 µg/ml for rosuvastatin.

Keywords: Teneligliptin, rosuvastatin, RP-HPLC, simultaneous estimation, validation, ICH guidelines.
How to cite this article: Kumari A, Kaura S. Development and validation of RP-HPLC analytical method for simultaneous estimation of Teneligliptin and Rosuvastatin in dosage form. Int J Drug Deliv Technol. 2026;16(53s): 590-595. DOI: 10.25258/ijddt.16.53s.63
Source of support: Nil.
Conflict of interest: None.

Introduction

Teneligliptin (TGL) (figure 1) is a novel new-generation dipeptidyl peptidase-4 (DPP-4) inhibitor used in treatment of Type 2 diabetes mellitus. Rosuvastatin (RVS) (figure 2) is a member of the HMG-CoA reductase inhibitors class of anti-hyperlipidemic agent.[2] The fixed dose combination of TGL and RVS is used to manage dyslipidemia that is characterized by high cholesterol levels in diabetic patients.

Several methods for individually estimating of TGL and RVS have been reported over years by several researchers. On the other hand only a handful of methods for simultaneous estimation of TGL and RVS in fixed are reported.[3-10] Also a few of these methods lack the data for system suitability and repeatability. The objective was the present work was to develop a simple high performance liquid chromatography (HPLC) method for simultaneous estimation of teneligliptin and rosuvastatin in oral fixed dose combination and validate the method as per ICH guidelines.

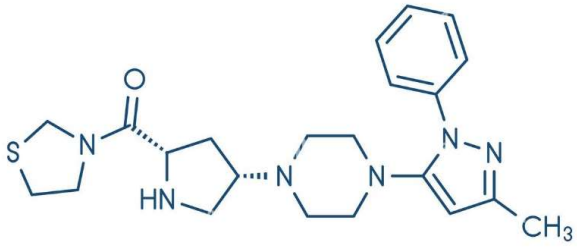

Figure 1. Chemical structure of teneligliptin

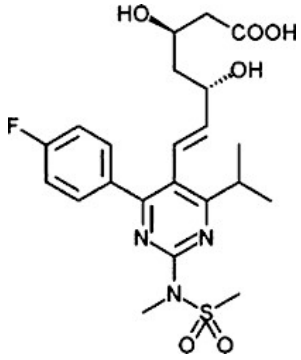

Figure 2. Chemical structure of rosuvastatin

Material and Methods

Teneligliptin (TGL) (>98%) and Rosuvastatin (RVS) (>98%) pure drugs were purchased from Yarrow Pharmaceuticals, Mumbai. All solvents used were of HPLC grade while the other reagents were of analytical grade. HPLC (Shimadzu, P series), electronic balance (Wensar), pH meter (Labtronics, LT-53), sonicator (Biotechnics) were used in the study.

Determination of solubility

The qualitative solubility of TLG and RVS was observed by dissolving a very small quantity of the individual drug in 1 mL of different solvents (water, methanol, acetonitrile).

Instrument Used for method development

A Shimadzu HPLC system (P-series) equipped with PDA detector (SPD-M40), pump (LC-20AD), column oven (CTO-10AS VP), C-18 column (Shim-pack Solar, 4.6/250 mm, 5µ) and autosampler (SIL-20AC HT) was used for simultaneous estimation.

Preparation of standard solution

A mixed standard stock solution of TGL and RVS was prepared by dissolving 10 mg each of both the drugs in 10 mL methanol. 2.0 mL of this stock solution was withdrawn and diluted with methanol (8 mL) to obtain a solution containing 200 µg/mL of TGL and RVS. 1.0 mL of the above solution was further diluted with methanol to produce working standard solution containing TGL (20 µg/mL) and RVS (20 µg/mL). The solution was sonicated for 10 min and filtered through 0.45 µ membrane filter before use.

Tablet sample for analysis

Ten tablets were weighed to determine the average weight. The tablets were crushed and finely powdered in a mortar using pestle. Tablet powder equivalent to 20 mg TGL and 10 mg RVS was accurately weighed and transferred to volumetric flask and dissolved in 10 mL methanol by sonication for 30 min. The solution was filtered through Watman filter paper and 2.0 mL of this solution was diluted suitably to obtain concentration of TGL (20 µg/mL) and RVS (10 µg/mL). The solution was sonicated for 10 min and filtered through 0.45 µ membrane filter syringe filter before use.

Optimized conditions

The optimized conditions for the simultaneous estimation of TGL and RVS include:

Column: Octadecylsilane (C_{18}) (4.6 x 250mm, 5µm, Shim-pack Solar)

Mobile Phase – Isocratic mixture of 1% Formic acid and acetonitrile (50:50), pH 2.65

Flow rate: 1.0 ml per min

Wavelength: 265 nm

Injection volume: 20 µl

Column Temperature: 40°C

Run time: 15 min

Validation of method[11,12]

The method was validated according to ICH Q2B guidelines for accuracy, precision, linearity, limit of detection, limit of quantification and robustness.

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Specificity

Solutions of standard and samples were prepared as per test procedure and injected into the HPLC system. The chromatograms were recorded. A study to establish the interference of blank was conducted by injected the mobile phase into HPLC system.

Linearity

The working standards were prepared at different concentrations by diluting with the diluents. The dilutions were injected in to the HPLC system and analyzed as per the optimized conditions.

Accuracy

Accuracy of the method was determined by recovery studies. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels (50%, 100%, 150%) of the target assay concentration. The amounts added, amounts estimated and the individual recovery and mean recovery values were calculated

Precision

Repeatability

The working standard solution was injected in six replicates in the HPLC system and the peak area was measured. The % RSD for the area was calculated.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was evaluated on different days by different analysts.

Limit of Detection and quantification

The LOD and LOQ were calculated using calibration curve method. The following formula was used.

LOD = 3.3 sigma/S

LOQ = 10 sigma/S

Where sigma is standard deviation of the calibration curve, S is the slope of the calibration curve

Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous batches by differing physical parameters like flow rate and mobile phase composition which may differ but the responses would be still within the specified limits of the assay.

Results and Discussion

The preliminary screening of both the drugs was done for solubility and the chromatographic conditions were selected by trial of various combinations maintaining pH form 2.5 to 4.0. The chromatogram was obtained using the optimized conditions (figure 3-5).

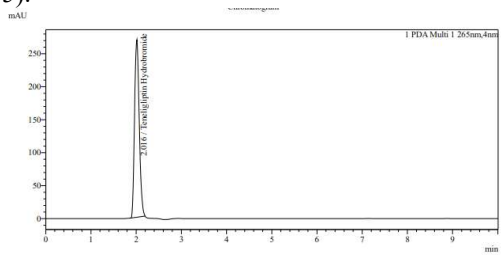

Figure 1. Chromatogram of Teneligliptin

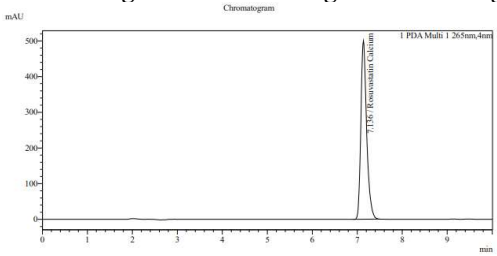

Figure 2. Chromatogram of Rosuvastatin

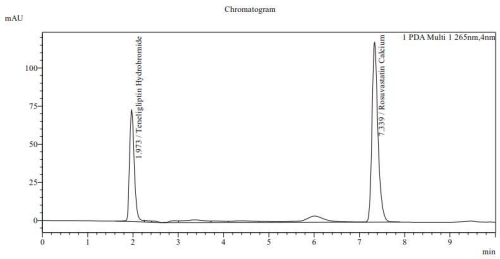

Figure 3. Chromatogram of TGL and RVS combination

While most of the previously reported methods utilized wavelength for detection as 240-245 nm based on the isosbestic point obtained from overlay spectra of the drugs[5,8,10], our method utilized 265 nm based on PDA result obtained at various wavelengths. At 240 nm the elution of teneligliptin occurred along with other impurities of the sample and hence it was not selected for estimation. All the methods used combination of buffer and organic solvent for elution. A few methods required very high amount of the buffer while one method reported use of more than three solvent in the mixture[5,6]. Our method on the other hand used 50% buffer and 50% organic solvent and would be the best possible method for the simultaneous estimation of TGL and RVS.

Validation of the method

System Suitability

The % RSD of retention time was less than 2% and tailing factor and number of theoretical plates were found to be satisfactorily within the limits for both TGP and RVS (table 1). Hence the selected system parameters were found to be suitable for the simultaneous estimation.

Table 1. System suitability parameters

System suitability parameters	TGL	RSD	RVS	RSD
Retention Time	2.027 ± 0.0035	0.1763	7.885 ± 0.0074	0.0953
Tailing Factor	1.277 ± 0.0341		0.99	0.956 ± 0.0439
No.of Theoretical plates	9397.66		153741.20	

Linearity

The linearity range was found to be from 6 to 30 µg/mL for both the drugs (Table 2).

Table 2. Linearity

Concentration (µg/ml)	AUC (TGL)	AUC (RVS)
6	16324.33	33553
12	29584.33	66949.67
18	47440.67	97111.33
24	65079.00	141831.00
30	84354.00	177927.70
Correl Coeff (r^2)	0.9961	0.9958
Slope (m)	2859.2	6060.5
Intercept (c)	-2909.7	-5614.7

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The spiked sample was recovered within a range of 98-102% justifying the accuracy of method in estimating the concentration of the drugs of mixture (Table 3).

Table 3. Accuracy

Conc. of preanalyzed TGL in µg/ml)	Conc. of TGL added to final (µg/ml)	% Recovered (mean)	Conc. of preanalyzed RVS µg/ml)	Conc. of RVS added to final (µg/ml)	% Recovered (mean)
12	6	98.88	12	6	100.77
12	12	98.12	12	12	99.25

Precision

The precision of the method depicts its ability to reproduce the results irrespective of the day of analysis, the analyst or even the instrument used for analysis. The results of repeatability and intermediate precision are reported in table 4 and table 5 respectively.

Table 4. Repeatability of the developed method

Concentration (µg/ml)	TGL (20 µg/mL)		RVS (20 µg/mL)	
	Retention time	Peak Area	Retention time	Peak Area
Mean (n =6)	1.973	519239.3	7.296	1076677
SD	0.000	7828.397	0.0310	2293.093
%RSD	0.000	1.507	0.425	0.212

Table 5. Intermediate precision

Concentration (µg/ml)	TGL (20 µg/mL)		RVS (20 µg/mL)	
	Retention time	Peak Area	Retention time	Peak Area
Mean (n =6)	1.973	519165.2	7.288	1076133
SD	0.0012	7874.06	0.0219	1834.09
%RSD	0.064	1.516	0.300	0.170

The results reveal that the % RSD in both the repeatability and intermediate precision studies was less than 2%, thereby ascertaining that the developed method will produced consistent results.

Robustness

The deliberate changes in flow rate and mobile phase composition were made in order to study the effect of the same on the results obtained by the method. The method was able to adjust to the changes with no significant change in the retention time of the eluted components (Table 6).

Table 6. Robustness of method

Flow rate	TGL		RVS	
	Retention Time	Peak Area	Retention Time	Peak Area
0.7	2.752	35277	10.347	44648
1.5	1.312	16416	4.939	33242
60FA:40ACN	2.155	226821	14.368	681619
40FA:60ACN	1.877	335802	4.437	289394

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the signal to noise ratio method. The LOQ and LOQ was found to

be 2.25 µg/mL and 6.84 µg/mL for TGL and 2.33 µg/mL and 7.08 µg/mL for RVS.

Application of the method to marketed formulation

The developed and validated method was applied for the analysis of the marketed formulation of TGL and RVS and the results obtained are presented in table 6. The percentage assay of TGL and RVS were found to be 100.15 % and 99.80 % respectively

Table 6. Results of assay of marketed formulation

Brand name & label content	Amount found (mg)*		Percentage recovery	
	TGL	RVS	TGL	RVS
Cedaglip-R 10 tablet	20.03	9.98	100.15	99.80

* Average of three replicate values

Conclusion

The investigation resulted in the development of a new RP – HPLC method for the simultaneous estimation of teneligliptin and rosuvastatin in tablet formulations. The method is simple, selective, reproducible and accurate with good precision and can be used for routine pharmaceutical analysis. The method could be easily used with accurate results for routine estimation of the above combination in dosage forms.

References

1. Teneligliptin Tablets. Summary of Product Characteristics. Available from https://rwandafda.gov.rw/wp-content/uploads/2022/11/TILYPTIN_20__TENELIGLIPTIN__TABLET-SMPC.pdf
2. Rosuvastatin calcium tablets. Highlights of Prescribing Information. Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021366s016lbl.pdf
3. Prasad Kumar L, Kabra P, Nargund SL, Gayathri A, Natikar A, Mundada J. A UV Spectroscopic Method for Simultaneous Estimation of Rosuvastatin and Teneligliptin from Bulk and Pharmaceutical Formulation. International Journal of Pharmaceutical Sciences Review and Research. 2025; 85(2): 122-126
4. Mandale DA, Shah C, Jatt R. Development and validation of novel RP-HPLC method for the simultaneous determination of Rosuvastatin and Teneligliptin in bulk and in synthetic mixture. International Journal of Pharmaceutical Research. 2021; 13(3): 498-506
5. Vyas AJ, Vadile HM, Godhaniya JP, Jadav CD, Patel AI, Patel AB, Dudhrejiya AV, Shah S, Chotaliya UJ, Sheth DB. RP-HPLC Method Development and Validation for Simultaneous Estimation of Rosuvastatin calcium and Teneligliptin hydrobromide hydrate in Synthetic Mixture. Research Journal of Pharmacy and Technology. 2024; 17(9):4325-8. doi: 10.52711/0974-360X.2024.00668
6. Patel S, Shah UA, Shah D, Dayaramani R. Stability indicating RP-HPLC method development for thesimultaneous estimation of teneligliptin and rosuvastatin in tablet dosage form. International Journal of Biology, Pharmacy and Allied Sciences. 2024; 13(7): 3246-3443
7. Dhoru MM, Parikh MP, Detholia KK, Patel PJ. Development and validation of rp-hplc method for simultaneous estimation of rosuvastatin and teneligliptin in their synthetic mixture. Indian Drugs. 2023; 60(2): 60-69. https://doi.org/10.53879/id.60.02.13015
8. Patel F, Dalwadi P, Patel D, Mehta N. Development And Validation of Rp-Hplc Method for Simultaneous Estimation of Teneligliptin Hydrobromide Hydrtae and Rosuvastatin Calcium in Synthetic Mixture. International Journal of All Research Education & Scientific Methods. 2021; 9(4): 2712-2719.
9. Patel R, Shah J, Patel M. Analytical Quality by Design Based HPLC for Quantitative Analysis of Teneligliptin and Rosuvastatin Calcium Tablets in the Presence of Force Degradation Products. Separation Science Plus. 2025; 8(1): e202400229. https://doi.org/10.1002/sscp.202400229
10. Mukhtiyarmiya SJ, Sarkar D. Stability indicating analytical method development and validation for simultaneous estimation of teneligliptin hemihydrate hydrobromide and rosuvastatin calcium in synthetic mixture. World Journal of Pharmacy and Pharmaceutical Sciences. 2021; 11(1): 1916-1952.
11. Biswas P, Jain P, Parkhe G, Mishra B. Validated HPLC method for the estimation of Aspirin and Omeprazole in dosage form.

Journal of Pharmacology and Biomedicine. 2018; 2(3): 171-180.

12. Patel A, Soni P, Yadav N, Omray LK. New cost effective RP-HPLC method development and evaluation for quantitative estimation of Pitavastatin in pharmaceutical formulation. Journal of Pharmacology and Biomedicine. 2018; 2(3): 181-188