Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 6
Open Access

Developing a Validated RP-HPLC Chromatographic Method for the Identification of Rosuvastatin and Teneligliptin and its related Impurities in Pharmaceutical Tablet DosageForm

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Cite this paper as: Dhwani Shah,Sejalben Patel,Ujashkumar Shah,Khushbu S. Patel,Richa Dayaramani (2024) Developing a Validated RP-HPLC Chromatographic Method for the Identification of Rosuvastatin and Teneligliptin and its related Impurities in Pharmaceutical Tablet Dosage Form. Frontiers in Health *Informatics*, *13(6)* 1158-1172

Abstract

Introduction: Diabetes and cholesterol are major global health issues, affecting millions worldwide. Diabetes, including type 1 (insufficient insulin) and type 2 (insulin resistance), is rising rapidly, contributing to severe complications and deaths. High cholesterol, often linked to lifestyle factors, increases the risk of cardiovascular diseases, necessitating effective management and prevention strategies.

Objectives: Reverse phase gradient high-performance liquid chromatography (RP-HPLC) is a simple, cost-effective, linear, accurate, and selective approach that has been developed and validated to assess the related impurities of Rosuvastatin and Teneligliptin in a combination of tablet dose form.

Methods: Analysis was conducted on a 250 mm x 4.6 mm, 5 µm Hypersil BDS C18 column using Acetonitrile (ACN) in Channel A and 1 % formic acid pH 4 in Channel B with 70:30 v/v ratio at a detection wavelength of 280 nm and a flow rate of 1 ml/min. The analytical method was validated according to ICH (International Council for Harmonisation) guidelines.

Results: The linearity of Rosuvastatin (25-75 µg/ml) with LOQ 0.51 µg/ml, Teneligliptin (12.5-37.5 µg/ml) with LOQ 0.258 and Teneligliptin Impurity (2.5-7.5 µg/ml) with LOQ 0.086 µg/ml. The correlation coefficient was consistently observed to be not less than 0.99 for all analytes. The % Recovery value was found to be 100.01% minimum and 100.38% maximum for Rosuvastatin, 100.25% minimum and 103.56% maximum for Teneligliptin and 98.25 % minimum and 101.16 % maximum for impurity A. Limit of Detection was found to be 0.17 µg/ml for Rosuvastatin, 0.085 µg/ml for Teneligliptin and 0.0286 µg/ml for Teneligliptin impurity A. The relative standard deviation value for repeatability, Interday precision and Intraday precision was less than 2%.

Conclusion: This RP-HPLC technique is reliable, efficient, and well-suited for accurately quantifying Rosuvastatin and Teneligliptin, including the detection of Teneligliptin Impurity A, in combined tablet

formulations.
Key word: Rosuvastatin, Teneligliptin, Impurity, Validation

Introduction

Approximately 460 million persons worldwide suffer with diabetes and high Cholesterol.This will increase to 700 million by 2045. In most nations, the percentage of individuals with type 2 diabetes (T2D) is rising. 4.2 million fatalities were related to diabetes and obesity is also increased over the world wide[1] Type 1 diabetes is caused by insufficient insulin production, but type 2 diabetes (T2DM) is brought on by cells that are resistant to insulin[2].By lowering blood glucose levels, anti-diabetic medications help to treat diabetes mellitus. Type 2 diabetes, which is characterized by polyphagia, polyuria, and polydipsia, requires a lifetime of anti-diabetic medication.[3-4] Lipoprotein disorders are clinically important due to the role of lipoproteins in atherogenesis and the associated risk of atherosclerotic cardiovascular disease[5-6] Lipoproteins are made up of lipids and protein, and as such, they can carry triglycerides, cholesterol, and fat-soluble vitamins to the appropriate organs when needed. Liposomal illnesses were formerly the purview of lipid specialists[7-8]. On the other hand, the advantage of statin medications, particularly in lowering cardiovascular (CV) events, has made treating hypercholesterolemia easier[9].

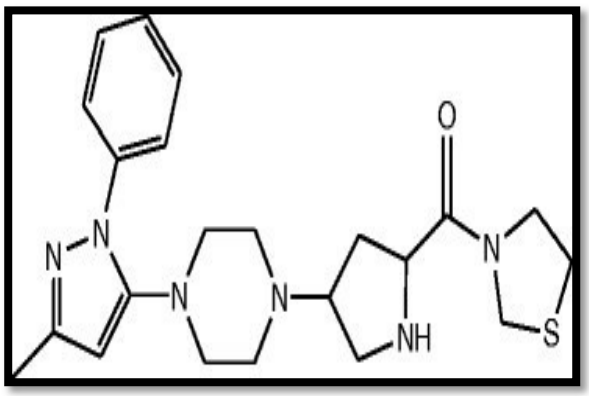
Rosuvastatin is chemically (3R,5S,6E)-7-[4-(4-Fluorophenyl)-2-(N methyl methane sulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.[10] (figure 1) Statins are medications that inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase[11], which is responsible for the production of cholesterol. They have been proven to effectively lower both total and LDL cholesterol levels[12]. Multiple large-scale randomized control trials have consistently shown that lowering LDL-C, especially with statins, significantly decreases the risk of cardiovascular deaths and events.[13] Studies have alsorevealed that statins not only lower cholesterol, but also improve endothelial function, increase the stability of atherosclerotic plaques, and suppress inflammatory and thrombogenicresponses in arterial walls[14]. These findings highlight the significant potential of statins in treating this condition[15]

Teneligliptin Chemically, [(2S, 4S)-4- [4-(5-methyl- 2phenylpyrazol-3-yl) piperazin-1-yl]pyrrolidin-2-yl] -(1, 3thiazolidin-3-yl) (Figure 2). Teneligliptin is a novel oral dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes mellitus (T2DM) having a unique structure characterized by five consecutive rings[16], which produce a potent and long-lasting effect[17].Teneligliptin is currently used in cases showing insufficient improvement in glycemiccontrol even after diet control and exercise or a combination of diet control, exercise, and oralhypoglycemic drugs used include Biguanides, Sulphonylureas[18-20]

Impurity profiling encompasses a range of analytical procedures aimed at detecting, identifying, and quantifying both known and unknown impurities (organic and inorganic, including residual solvents) in bulk drugs and pharmaceutical formulations[21]. It serves as a vital means to assess the quality, safety, efficacy, and stability of these substances[22], thus representing a cornerstone of modern drug analysis. Particularly pivotal during drug synthesisand formulation, impurity profiling yields essential insights into the quality, safety, efficacy, and toxicity of drugs, as well as various limits of detection and quantification[23]. It also provides structural elucidation for numerous organic and inorganic impurities commonly associated with bulk drugs and finished products[24].

In ensuring quality control, it's crucial to develop analytical methods for combination products and their impurities[25-27]. Various advanced techniques exist in literature for analyzing Rosuvastatin and Teneligliptin, either separately or combined with other drugs, such as UV spectrometry, HPLC, and stability-indicating HPLC methods[28]. However, there's currently no RP-HPLC

stability-indicating chromatographic method available for determining Rosuvastatin and Teneligliptin with their related impurities in the dosage form.[29-39] Therefore,a precise, accurate, and sensitive stability-indicating chromatographic method for determining Rosuvastatin and Teneligliptin with their related impurities was developed and validated according to the Q2 (R1) guideline.



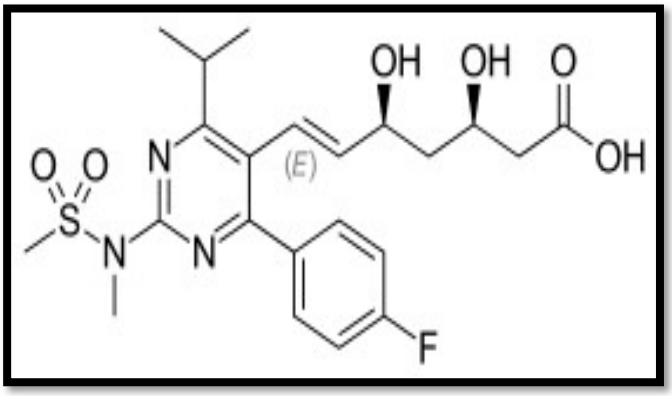


Fig.1 Structure of Teneligliptin **Fig.2 Structure of Rosuvastatin**

OBJECTIVES

Materials

The reference standards for Rosuvastatin and Teneligliptin and impurity A, were generously provided by Emcure Pharmaceuticals Limited, located in Ahmedabad. Solvents and reagents used in this study included methanol, water, HPLC-grade acetonitrile, formic acid and analytical reagent-grade orthophosphoric acid, all of which were sourced from Finar Mumbai. Additionally, a commercial tablet formulation, Cedaglip R, containing Rosuvastatin(20 mg) and Teneligliptin (10 mg), produced by Simpex Pharma Pvt. Ltd., was acquired fromthe local market for analysis.

Instrumentation

For the method development, various instruments were utilized including the Shimadzu LC- 20 AT HPLC chromatographic system, Shimadzu digital weighing balance (model ATX 224), a pH meter from Lab Scientific Pvt. Ltd, a Frontline Ultrasonic Cleaner ultrasonicator,a hot air oven from India, and a Thermolab unit from Mumbai. Filtration was performed using a 0.45µ Millipore filter**.**

METHODS

Chromatographic Condition

The separation of Rosuvastatin and Teneligliptin was successfully performed using an ECO- C18 5µ (15mm*4.6mm*5µ (particle size)). The mobile phase consisted of Channel A ACN (Acetonitrile) and Channel B 1% Formic acid pH 4, 70:30 v/v at a flow rate of 1 mL/min.The injection volume was 20.0 µL, and the detection was carried out at a wavelength (λ max) of 280 nm over a runtime of 15 minutes.

Preparation of Mobile Phase

Channel A contain Acetonitrile and Channel B contain 1% Formic acid (1 ml Formic acid in 100 ml water) and pH 4 adjusted with ortho phosphoric acid in a ratio of 70:30 % v/v

Standard Solution Preparation

Standard stock solution of Rosuvastatin (200 mcg/ml)

Weighed accurately about 20 mg of Rosuvastatin, transferred in to 100 ml of volumetric flaskand make up the volume up to 100 ml with solvent to get final concentration 200 mcg/ml of Rosuvastatin.

Rosuvastatin Working Standard Solution (50 mcg/ml)

Pipette out 5 ml from standard stock solution of rosuvastatin and transferred into 20 ml of volumetric flask and volume make up to 20 ml with solvent to get final concentration of Rosuvastatin is 50 mcg/ml.

Standard stock solution of Teneligliptin (100 mcg/ml)

Weighed accurately about 10 mg of Teneligliptin transferred in to 100 ml of volumetric flask and make up the volume up to 100 ml with solvent to get final concentration 100 mcg/ml of Teneligliptin.

Teneligliptin Working Standard Solution (25 mcg/ml)

Pipette out 5 ml from standard stock solution of teneligliptin and transferred into 20 ml of volumetric flask and volume made up to 20 ml with solvent to get final concentration of Teneligliptin is 25 mcg/ml.

Standard stock solution of Impurity A (100 mcg/ml)

Weighed accurately about 10 mg of impurity A, transferred in to 100 ml of volumetric flask and make up the volume up to 100 ml with solvent to get final concentration 100 mcg/ml of Impurity A.

Impurity A Working Standard Solution (5 mcg/ml)

From standard stock solution of impurity, a pipette out 1 ml and transferred in to 20 ml of volumetric flask and volume make up to 20 ml with solvent to get final concentration of impurity A is 5 mcg/ml working standard solution.

Preparation of Sample Solution from Pharmaceutical Marketed TabletsStock solution

About 10 tablets of Cedaglip R were weighed, and an average weight of 10 tablets was determined and powdered finely in a mortar. Powdered tablet equivalent to 20 mg of Rosuvastatin and 10 mg of Teneligliptin was accurately weighed and transferred into 100 ml volumetric flask, sonicate for 10 min using 60 ml of mobile phase after confirming complete solubilization of drugs volume made up to the marks and filtered through 0.45 µ membrane filter, filtrate was collected. From above filtrate pipette out 5 ml and transferred in to 20 ml ofvolumetric flask and volume made up to the mark with mobile phase to get final test solution

Chromatographic Separation

Standard solutions of Rosuvastatin and Teneligliptin, along with Teneligliptin impurity (Impurity A), were administered into the column using a 20 µL micro-syringe. The chromatographic run was conducted for the necessary duration, with detection occurring at a wavelength of 280 nm. The chromatogram was terminated once complete separation was achieved. Data pertaining to resolution, retention time, and peak characteristics such as heightand area were recorded using the Lab-solution software.

Method Validation

The proposed method was validated in accordance with ICH guidelines Q2 (R1)[34]

Validation covered various parameters including accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness.

System Suitability

System suitability parameters like retention time, theoretical plates, resolution tailing factors were calculated

Specificity

The impact of excipients and additives in tablets was studied, and the RP-HPLC method's specificity was confirmed by testing blank and placebo solutions.

Linearity

The linearity of an analytical method is assessed by determining how closely a calibration curve, which plots response against concentration, aligns with a straight line. In this experiment, a calibration curve was prepared

using a stock solution containing 200 µg/ml of Rosuvastatin, 100 µg/ml of Teneligliptin and 100 mcg/ml Impurity A. Aliquots of 2.5 ml, 4 ml, 5 ml, 6 ml, and 7.5 ml were transferred into 20 ml volumetric flasks, sonicated, and then diluted to the mark with a suitable diluent. This process produced solutions with concentrations of 25, 40, 50, 60, and 75 ppm for Rosuvastatin and 12.5, 20, 25, 30, and 37.5 ppm for Teneligliptin and for impurity A aliquot of 0.5, 0.8,1,1.2 and 1.5 ml were transferred into 20 ml of volumetric flask and diluted up to the mark with solvent to get final concentration 2.5, 4, 5, 6 and 7.5 ppm for Impurity A. A 20 µL aliquot from each prepared solution was injected into the chromatography system under predefined operational conditions. The resulting calibration curve was constructed by plotting the peak areas against the concentrations, and a regression equation was derived. Each plotted response represented the average of three determinations to ensure accuracy.

Precision

System precision was evaluated by performing six injections of a standard solution containing Rosuvastatin (50 µg/mL), along with Teneligliptin (25 µg/mL) and its Impurity A (5 µg/mL). The resulting chromatograms were analysed and peak areas recorded to assess repeatability.

For precision testing, a standard solution with concentrations of Rosuvastatin at the limit of quantitation (LoQ), 50 µg/mL, Teneligliptin 25 µg/mL and its Impurity A 5 µg/mL were used. These solutions were tested for Interday precision by analysing them on different days, and for Intraday precision by analysing them multiple times on the same day. From these tests, the percent relative standard deviation (%RSD) was calculated to determine the precision of the method.

LOD and LOQ

The Limits of Detection (LoD) and Limits of Quantification (LoQ) for both the drugs and their impurities were determined based on the data obtained from linearity studies. Subsequently, the LoQ and LoD were computed using the following formula:

LoQ = 10*Standard Deviation/Slope of Calibration curve LoQ= 3.3* Standard Deviation/Slope of Calibration curve

Accuracy

To verify the accuracy of the proposed method for determining Rosuvastatin, Teneligliptin and Impurity A, recovery studies were conducted at LOQ, 80%, 100%, and 120% of the test concentration following ICH guidelines, with each level tested three times.

Robustness

The robustness study was conducted under chromatographic conditions to assess the impactof minor variations as outlined in the Chromatographic Conditions section. This study focused on factors that were identified as critical sources of variability in the operating procedures. Specifically, adjustments included altering the mobile phase ratio by ±2 mL, pH of mobile phase ±0.2 and modifying the flow rate of the mobile phase by ±0.2 mL/min. Throughout these experiments, the composition of the mobile-phase components remained unchanged. The effects of these alterations were then evaluated in terms of their impact onthe system suitability for standard preparation.

Result and Discussion System Suitability Parameter

System Suitability was assessed using various parameters such as retention time, theoretical plates, resolution, and tailing factor. The purpose of evaluating system suitability was to ensure the repeatability and resolution of the system were adequate for the intended analysis. The system suitability parameters recorded for Rosuvastatin, Teneligliptin and Impurity A arepresented as follows:

Table 1 System Suitability Parameter

Parameters	Rosuvastatin	Teneligliptin	Impurity A
Retention time	5.37	8.80	13.7
Theoretical plates	3440	5290	3562
Tailing factor	1.26	1.269	1.239
Resolution	--	8.07	7.32

Specificity

The specificity of the method was confirmed by analysing the resolution factor between the drug peaks and their nearest resolving peaks, as well as among all other peaks. This was doneto ensure clear separation of Rosuvastatin, Teneligliptin and Impurity A were compared with the chromatograms of blank samples of Rosuvastatin and Teneligliptin. No interference was observed in the chromatograms of the drugs and their impurities with those of the blanks, confirming the specificity of the developed chromatographic method, shown in figure 3 and 4and Figure 5 Shows Peaks of Impurity A.

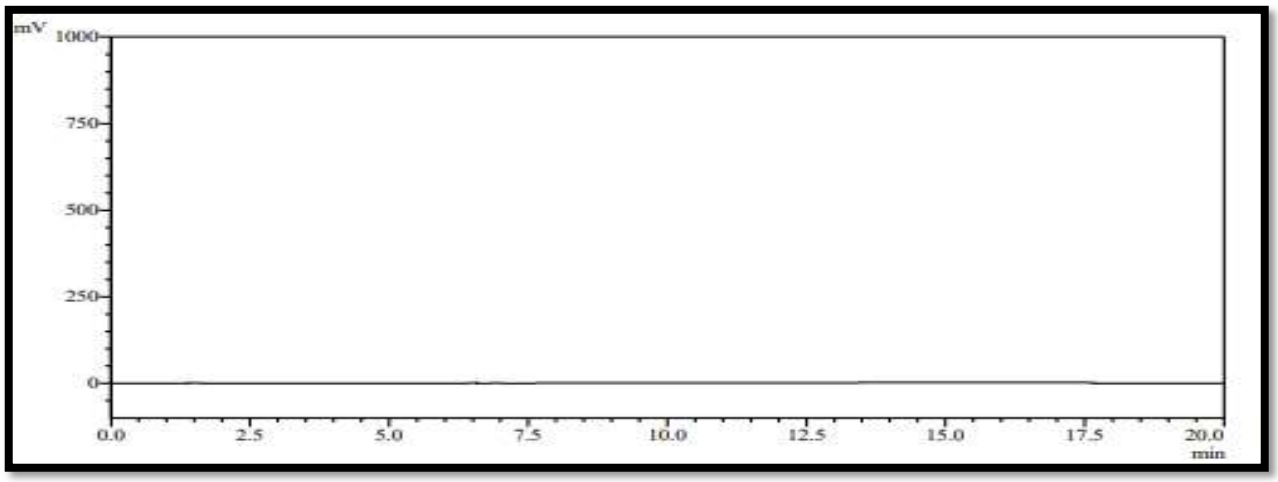


Fig 3. Blank Chromatogram of Rosuvastatin and Teneligliptin

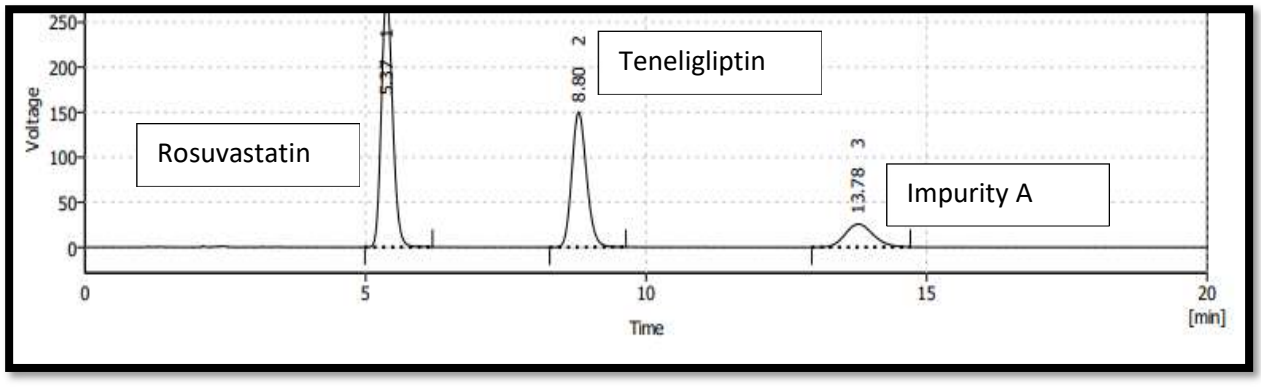


Fig 4. Sample Chromatogram of Rosuvastatin and Teneligliptin

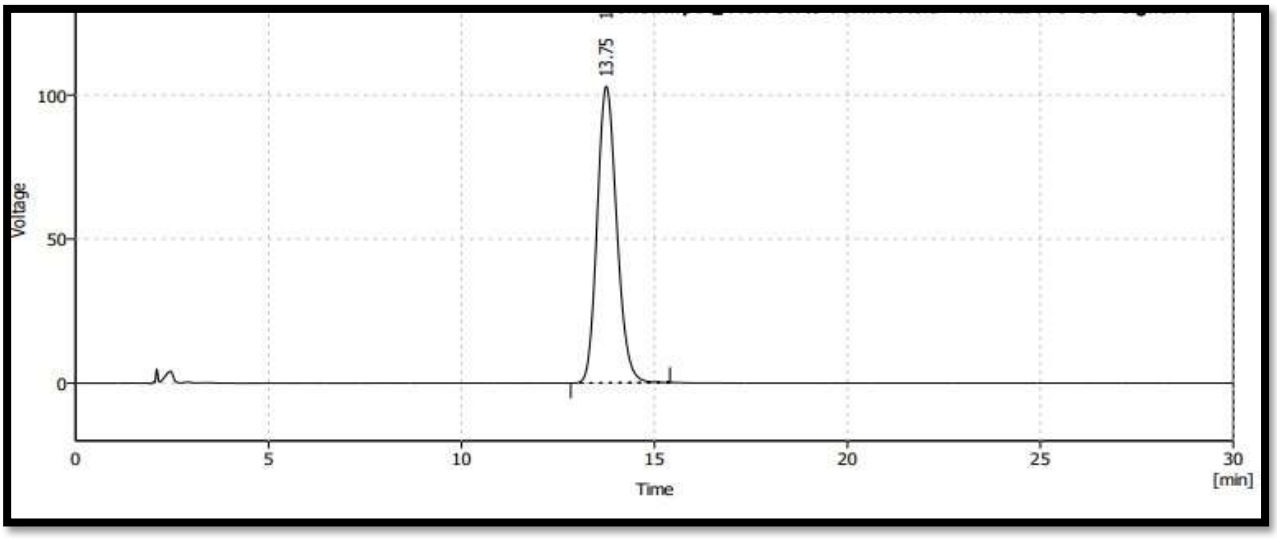


Fig 5. Chromatogram of Impurity A

Linearity

The linearity of Rosuvastatin, Teneligliptin and Impurity A was evaluated by analysing a combined standard solution within the ranges of 25 to 75 µg/mL, 12.5 to 37.5 µg/mL and
2.50 to 7.50 µg/mL respectively. The correlation coefficient for the calibration curve of Rosuvastatin, Teneligliptin and Impurity A was determined to be not less than 0.999 for each compound. Calibration curve of Rosuvastatin, Teneligliptin and Impurity A shown in figure no 6,7 and 8 and % RSD shown in table no 2

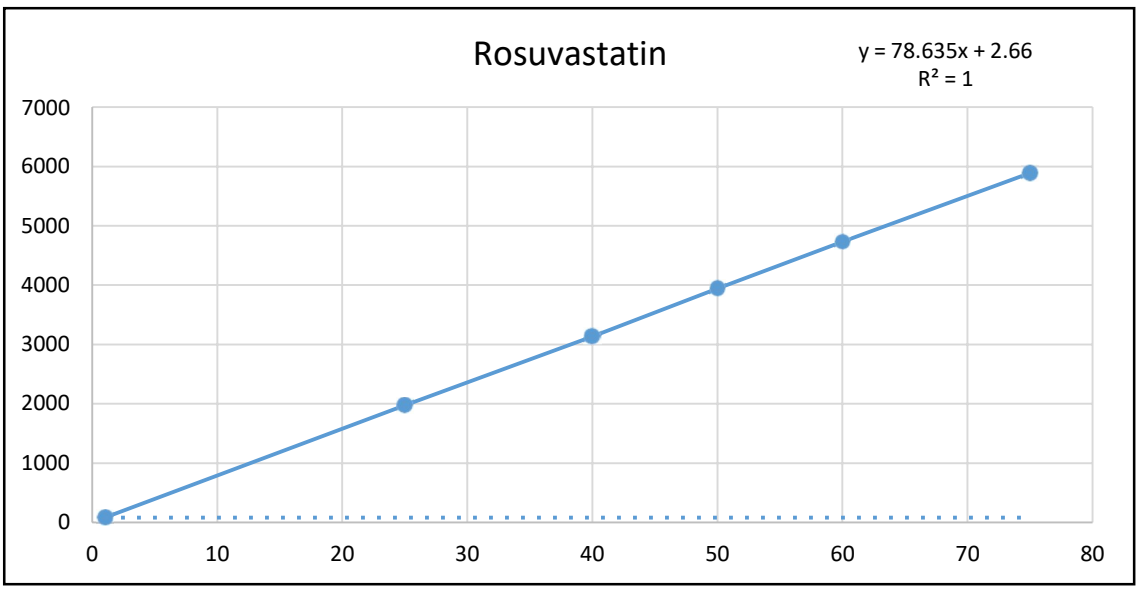


Fig 6. Calibration curve of Rosuvastatin

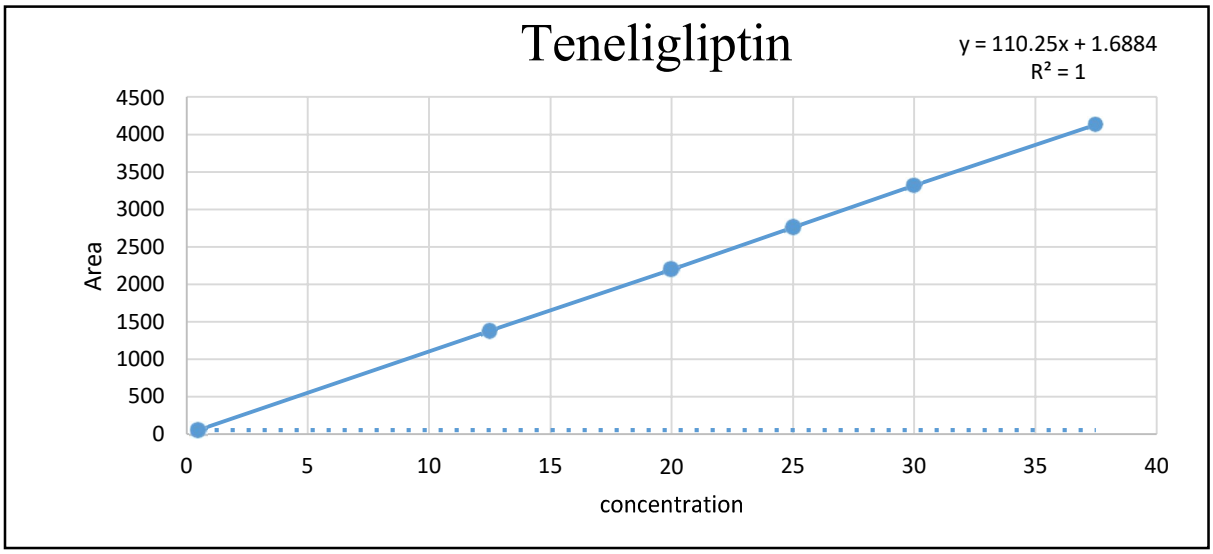


Fig 7. Calibration curve of Teneligliptin

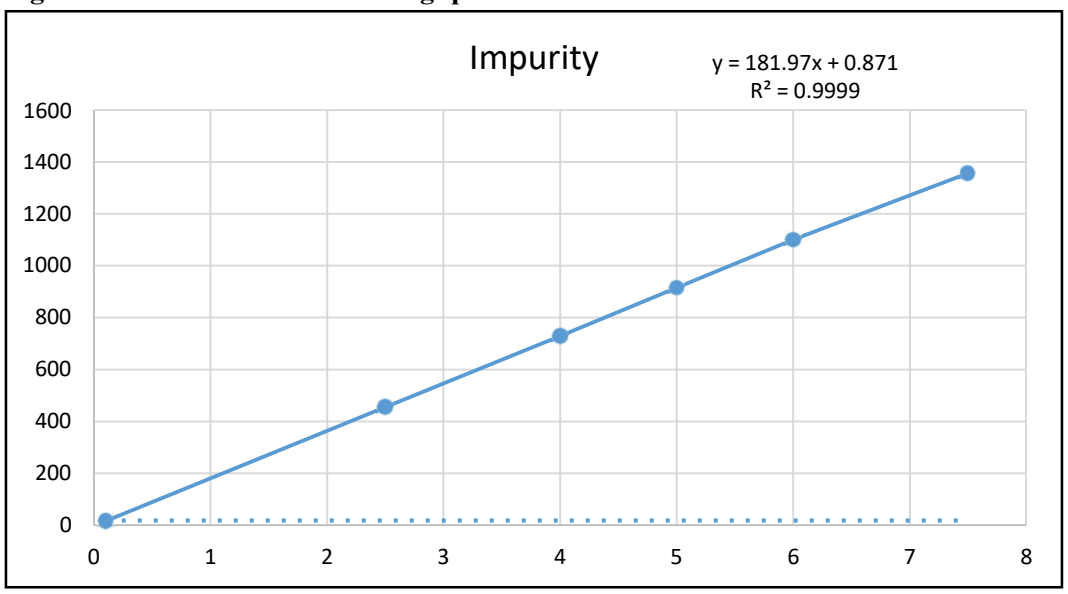


Fig 8. Calibration curve of Impurity A

Table 2 Analytical data of linearity

Rosuvastatin		Teneligliptin		Impurity A	
Concentration (µg/ml)	**Area ± S. D**	**Concentration (µg/ml)**	**Area ± S. D**	**Concentration (µg/ml)**	**Area ± S. D**
25	1972.709±4.96	12.5	1382.675 ± 2.17	2.50	455.586±0.68
40	3136.557±2.76	20	2198.617±1.56	4.00	728.542±0.09

50	3940.866±0.55	25	2762.231±0.58	5.00	915.487±0.03
60	4733.184±0.61	30	3317.872±1.15	6.00	1099.755±0.05
75	5890.805±0.40	37.5	4129.373±1.01	7.50	1357.096±0.58
SD	10.48	**SD**	7.31	**SD**	6.19
Correlation Coefficient (r)	1	**Correlation Coefficient (r)**	1	**Correlation Coefficient (r)**	0.99

Precision Repeatability

The repeatability data for peak area measurement of Rosuvastatin, Teneligliptin and Impurity A was assessed based on six measurements of the same solution. The mean peak area observed was 3993.98 for Rosuvastatin, 2799.38 for Teneligliptin and 919.50 for Impurity A with % RSD 0.18,0.184 and 0.19 Respectively. These %RSD shown in table no 3 values fall well within the acceptance limit of not more than (NMT) 2%, demonstrating good repeatability.

Table 3 Analytical Data for Repeatability

Interday and Intraday precision

The Intraday and Interday precision data for Rosuvastatin, Teneligliptin and Impurity A are presented in Table no 4 and 5. The calculated %RSD values are all within the acceptance limits, indicating that the method is precise. Results were shown in table no 4 and 5.

Table 4 Analytical Data for Intraday

Rosuvastatin				Teneligliptin			Impurity A		
Sr no.	**Conc. (µg/ml)**	**Area Mean ± S. D (n=3)**	**% RSD**	**Conc (µg/ml)**	**Area Mean ± S. D (n=3)**	**% RSD**	**Conc (µg/ml)**	**Area Mean ± S. D (n=6)**	**% RSD**
1	25	1965.043±2.74	0.14	12.5	1383.82±2.71	0.19	2.5	455.23 ±0.39	0.087
2	50	3920.68 ±6.99	0.17	25	2742.38 ± 11.98	0.43	5	902.65 ±2.85	0.31
3	75	5788.57 ±9.27	0.16	37.5	4053.79 ±20.02	0.49	7.5	1333.62±2.28	0.17

Table 5 Analytical Data for Interday

Rosuvastatin	Teneligliptin	Impurity A

r no.	onc.	Area Mean ± S. D (n= 3)	RSD	r no.	onc	Area Mean ± S. D (n= 3)	RSD	r no.	onc.	Area Mean ± S. D (n =6)	RSD
	5	2019.92±3.28	.16		2.5	1424.81±2.31	.16		.5	468.64±0.76	.16
	0	3989.040±74.08	.85		5	2804.68±53.86	.92		5	923.88±15.14	.64
	5	5756.78±25.74	.447		7.5	4034.058±13.99	.34		.5	1328.15±5.63	.42

LOD and LOQ

The limits of detection (LOD) and limits of quantitation (LOQ) for both drugs were estimatedusing the linearity data depicted in Figures 6, 7 and 8. The calibration curve was repeated fivetimes, and the standard deviation of the intercepts was calculated. The LOD for Rosuvastatin was determined to be 0.17 µg/mL for Teneligliptin, it was 0.085 µg/mL and for its ImpurityA was 0.0286. The LOQ for Rosuvastatin, teneligliptin and its Impurity A was 0.51, 0.256 and 0.086 respectively. The results are presented in Tables 6.

Table 6 LOD and LOQ data for Rosuvastatin, Teneligliptin and Impurity A

Drug Name	LOD	LOQ
Rosuvastatin	0.17 µg/ml	0.51 µg/ml
Teneligliptin	0.085 µg/ml	0.258 µg/ml
Impurity A	0.0286 µg/ml	0.086 µg/ml

Accuracy

To assess the accuracy of the proposed method for determining Rosuvastatin, Teneligliptin and Impurity A, recovery studies were performed at LOQ, 80%, 100%, and 120% of the test concentration following ICH guidelines. The method's accuracy was confirmed by recovery studies from marketed formulations at three levels of standard addition. The percentage recovery ranged from 100.01 % to 100.38 for Rosuvastatin, 99.93% to 103.56% for Teneligliptin and 98.25 % and 101.16% for Impurity A table no 7.

Table 7 Analytical data for Accuracy

Drug	% Level	Amount of sample taken	Amount of standard recovery (mg)	% Recovery ± SD	%RSD

		(mg)			
Rosuvastatin	80 %	40 mg	40.12 mg	100.03% ±0.54	0.017
	100 %	50 mg	50.05 mg	100.01% ± 0.44	0.011
	120 %	60 mg	60.23 mg	100.38% ±0.47	0.0099
Teneliglipti n	80 %	20 mg	20.05 mg	100.25% ±0.18	0.0081
	100 %	25 mg	25.89 mg	103.56% ± 0.46	0.0166
	120 %	30 mg	29.98 mg	99.93% ± 080	0.0241
Impurity A	80 %	4 mg	3.93 mg	98.25% ± 0.50	0.068
	100 %	5 mg	4.98 mg	99.6% ± 0.44	0.048
	120 %	6 mg	6.07 mg	101.16% ±0.64	0.058

Robustness

The robustness study assessed the influence of small, deliberate variations in thechromatographic conditions. Specifically, the ratio of the mobile phase was altered by ±2 mL,flow rate of the mobile phase was adjusted by ±0.2 mL/min, and pH of Mobile phase was adjusted to ±0.2 mL/min without changing the components of the mobile phase. The effectsof these changes were observed on the system suitability for standard preparation. The resultsindicated that the changes were within the acceptance criteria, with % RSD values remaining within the standard limit of not more than 2% Shown in Table no 8

Table 8 Robustness Data of Rosuvastatin, Teneligliptin and Impurity A

Drug	**Variation**		**Mean area ±SD (n=3)**	**%RSD**
Rosuvastatin	Flow rate	0.8 ml/min	3920.24 ± 1.47	0.038
		1.2 ml/min	3922.96 ± 2.51	0.064
	Mobile Phase	68:32	3921.23 ± 1.22	0.031
		28:72	3921.41 ± 0.79	0.020
	pH	4.2	3922.09 ± 0.67	0.017
		3.8	3924.88 ± 1.54	0.039
Teneligliptin	Flow rate	0.8 ml/min	2762.89 ± 1.51	0.055
		1.2 ml/min	2764.33 ± 4.15	0.150
	Mobile Phase	68:32	2760.08 ± 0.47	0.017
		28:72	2761.042 ±	0.029

			0.79	
	pH	4.2	2761.25 ± 0.95	0.034
		3.8	2761.52 ± 1.12	0.041
Impurity A	Flow rate	0.8 ml/min	910.73 ± 0.64	0.071
		1.2 ml/min	912.91 ± 0.31	0.035
	Mobile Phase	68:32	912.38 ± 0.15	0.017
		28:72	913.06 ± 0.14	0.016
	pH	4.2	910.77 ± 0.57	0.063
		3.8	912.63 ± 0.54	0.060

Table 9 Summary of Validation data

Parameter	Rosuvastatin	Teneligliptin	Impurity A
ıearity (RegrassionValue)	25-75 µg/ml (1)	12.5-37.5 µg/ml (1)	2.5 to 7.5 µg/ml(0.99)
Repeatability(%RSD, n=6)	0.185	0.184	0.19
Precision (RSD) Intra-day (n=3) Inter-day (n=3)	0.14-0.17 0.16-1.85	0.19-0.49 0.16-1.92	0.087-0.31 0.17-1.64
Limit of Detection	0.17	0.085	0.028
Limit of Quantification	0.51	0.25	0.086
Robustness	Robust	Robust	Robust

Known and Unknown Impurities of Rosuvastatin and Teneligliptin

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Cedaglip R. The results of known and unknown impurities are calculated in %RSD. The % RSD Teneligliptin impurity A observed 0.19%. The % RSD values observed within standard limit of not more than 5%. The results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries. RP HPLC method was validated as per ICH guidelines. The

developed method was found to be linear within the range Correlation co-efficient for calibration curve of Rosuvastatin, Teneligliptin and Impurity A found to be NLT 0.999 respectively. The accuracy of method was determined at 80%, 100%, 120% level. ThePercentage recovery for Rosuvastatin was 100.01 to 100.38 %, for Teneligliptin 99.93 % to
103.56 % and for Impurity A 98.25 % to 101.16%. The LOD for Rosuvastatin was found
0.17 µg/ml, for Teneligliptin 0.085 µg/ml and for Impurity A 0.0286 µg/ml. Also, the LOQof Rosuvastatin was found by 0.51 µg/ml, for Teneligliptin LOQ was found to be 0.258 µg/ml and for Impurity A 0.086µg/ml indicates the sensitivity of the method. The developed method was found to be precise as the % RSD values for intraday and inter-day were foundto be less than 5.0%. The method was also found to be robustness indicated by the % RSD values which are less than 5 %.

Conclusion

There is no available analytical work regarding the related impurities RP-HPLC method for Rosuvastatin and Teneligliptin in the literature. Data on the behaviour of these drugs andtheir related impurities under chromatographic conditions and other relevant analytical properties are lacking. This study represents a novel attempt to develop and validate a related impurities method using RP-HPLC. The RP-HPLC method described here is specific, sensitive, rapid, and easy to perform, allowing for the simultaneous estimation of Rosuvastatin, teneligliptin, and their related impurity. This method achieves good separation and resolution of the chromatographic peaks of Rosuvastatin, teneligliptin, and their related impurity. The mobile phase used was Channel A ACN: Channel B 1% formic acid pH 470:30 v/v. The sample recoveries from all formulations matched their respective label claims,indicating no interference from formulation excipients in the estimation. The method was successfully validated in terms of specificity, precision, linearity, and robustness according toICH guidelines. It can be concluded that this method is suitable for routine analysis of related impurities of Rosuvastatin and Teneligliptin in combined dosage forms using RP-HPLC.

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