Research Article

A UV Spectroscopic Method for Simultaneous Estimation of Rosuvastatin and Teneligliptin from Bulk and Pharmaceutical Formulation

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ABSTRACT

A simple and precise UV spectroscopic method was developed for the simultaneous estimation of Rosuvastatin Calcium and Teneligliptin Hydrobromide Hydrate using distilled water as a solvent. The simultaneous estimation was carried out by developing an Absorbance Correction Method wherein at 243 nm both the drugs show good absorbance whereas at 310 nm only Rosuvastatin shows absorbance. Rosuvastatin was found to be linear in the concentration range of 3-25 µg/ml at 243 nm and 10-120 µg/ml at 310 nm and Teneligliptin was found to be linear in the concentration range of 5-35 µg/ml at 243 nm. The mean % assay of Rosuvastatin and Teneligliptin in marketed formulation was found as of 99.59 % and 100.21 %, respectively. LOD and LOQ for Rosuvastatin was found to be 1.095 and 3.320 µg/ml, and for Teneligliptin it was found to be at 0.535 and 1.622 µg/ml. The results % Recovery for Rosuvastatin and Teneligliptin were observed in the range of 98.07-101.12 % and 99.07-100.43 % respectively. % RSD for precision study was found less than 2% for both the drugs. The developed method can be used for the simultaneous estimation of Rosuvastatin and Teneligliptin from bulk and pharmaceutical formulation.

Keywords: Rosuvastain calcium, Teneligliptin hydrobromide hydrate, Validation, UV Spectroscopic Method, Absorbance Correction Method.

INTRODUCTION

Dyslipidemia can be characterized by an elevated level of TC (Total Cholesterol), LDL-C (Low-Density Lipoprotein Cholesterol), TGs (Triglycerides) and a lowered level of HDL-C (High-Density Lipoprotein Cholesterol) in the blood plasma. In other words, they indicate metabolic disturbances associated with the lipid profile. The prevalence of Dyslipidemia has increased over the past few years, and it often indicates the onset of cardiovascular diseases. Dyslipidemia often exhibits no peculiar symptoms and as such goes unnoticed in majority of the population. "Diabetic Dyslipidemia" refers to elevated levels of TGs, TRLs (Triglyceride-Rich Lipoproteins), and LDL-C while the level of HDL is lowered. [1]

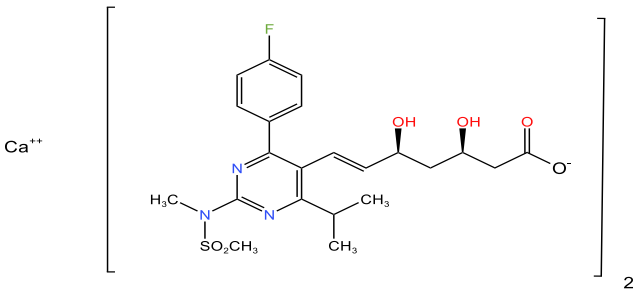

Figure 1: Structure of Rosuvastatin Calcium [3]

Rosuvastatin Calcium (ROSU) is a synthetic lipid-lowering drug that competitively inhibits 3-Hydroxy-3-MethylGlutaryl Coenzyme A (HMG CoA) reductase, a rate-limiting enzyme in the conversion of HMG-CoA to Mevalonate, which is a precursor of Cholesterol. Clinical studies have demonstrated Rosuvastatin to be effective in reducing LDL-C, the undesirable type of cholesterol that contributes to atherosclerosis and heart disease.[2]

Teneligliptin Hydrobromide Hydrate (TENA) is a Dieptidyl Peptidase 4 (DPP-4) inhibitor which is a relatively new class of anti diabetic drugs. It was introduced in India in May 2015 and it gained a lot of attention by becoming the most widely prescribed DPP-4 inhibitor within 8-9 months of its launch in the country. It has a unique chemical structure characterized by five consecutive rings (J-shaped), which might account for its unique potency and half-life time[4]

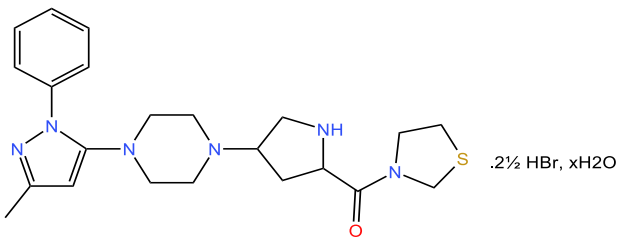

Figure 2: Structure of Teneligliptin Hydrobromide Hydrate[5]

The literature survey revealed several analytical methods, such as UV-visible spectrophotometry[6-12], Complexometric titration[13], Gas chromatography[14], HPLC[15-18], HPTLC[19], Stability Indicating HPTLC[20] and Colorimetric[21-22] for the estimation of Rosuvastatin calcium and Teneligliptin hydrobromide hydrate either as individual drugs or in combination with other compounds. However, there

remains a need to develop simple and rapid spectrophotometric method to estimate these two drugs in combination.

MATERIALS AND METHODS

Instruments:

- UV - Visible Spectrophotometer (Shimadzu – 1700, Software Version – UV Probe 2.32)
- Electronic Weighing Balance (Sartorius- TE-214S)
- Ultrasonicator (RC Systems – MU1700)

Chemicals:

Rosuvastatin Calcium and Teneligliptin Hydrobromide Hydrate were received as a gift sample from Glenmark Pharmaceuticals Ltd R&D Centre, Sinnar, Nashik, Maharashtra. Whereas the marketed formulation Cedaglip - R10 (ROSU 10 mg and TENA 20 mg) was received as a gift sample from Chemo Biological, New Delhi - 110 015.

Preparation of Standard Stock Solution

I. Standard Stock Solution of Rosuvastatin Calcium (ROSU)

An accurately weighed 20 mg of standard ROSU was transferred to 100 ml volumetric flask. It was dissolved in 50 ml distilled water by gentle shaking and volume was made up to the mark with distilled water to obtain final concentration of 200 µg/ml and labeled as 'Stock ROSU-A'.

II. Standard Stock Solution of Teneligliptin Hydrobromide Hydrate (TENA)

An accurately weighed 10 mg of standard TENA was transferred to 10 ml volumetric flask. TENA was dissolved in 5 ml distilled water by gently shaking and volume was made up to the mark with distilled water to obtain final concentration of 1000 µg/ml this was labeled as 'Stock TENA -A'. From the 'Stock TENA -A' (1000 µg/ml), 2.5 ml of aliquot was transferred to 25 ml of volumetric flask and volume was made up to the mark using distilled water. This solution was labeled as 'Stock TENA-B' (100 µg/ml).

Selection of Analytical Wavelength

From the 'Stock ROSU-A' (200 µg/ml) solution 0.5 ml of aliquot was transferred into a 10 ml volumetric flask and the volume was made up to the mark with distilled water to obtain the final concentration of 10 µg/ml. The solution was scanned in the spectrum mode from 200 nm to 400 nm.

From the 'Stock TENA-B' (100 µg/ml) solution 2 ml of aliquot was pipetted out into 10 ml volumetric flask and the volume was made up to the mark with distilled water to get the concentration of 20 µg/ml. The solution was scanned in the spectrum mode from 200 nm to 400 nm. Results are shown in Figure 3.

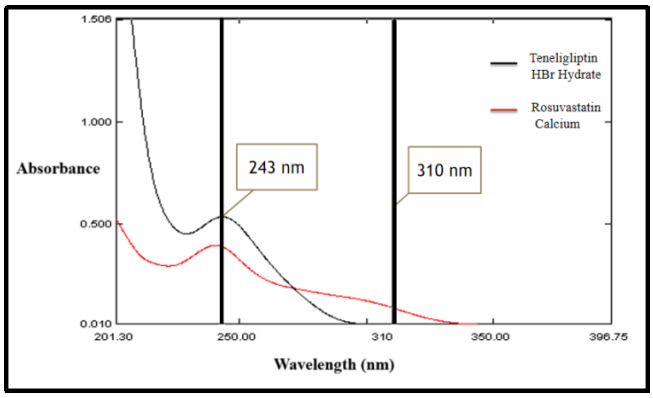

Figure 3: Overlain Spectra of ROSU (10 µg/ml) and TENA (20 µg/ml) in Distilled Water

Calibration Curves for ROSU and TENA

The stock solutions of ROSU was diluted to get concentration range of 10-120 µg/ml and 3-25 µg/ml and absorbance of these solutions were measured at 310 and 243 nm respectively. While for TENA the stock solution was diluted to get the concentration range of 5-35 µg/ml and absorbance of these solutions were measured at 243 nm. Calibration curves were plotted for these drugs as shown in the Figure 4, 5 and 6.

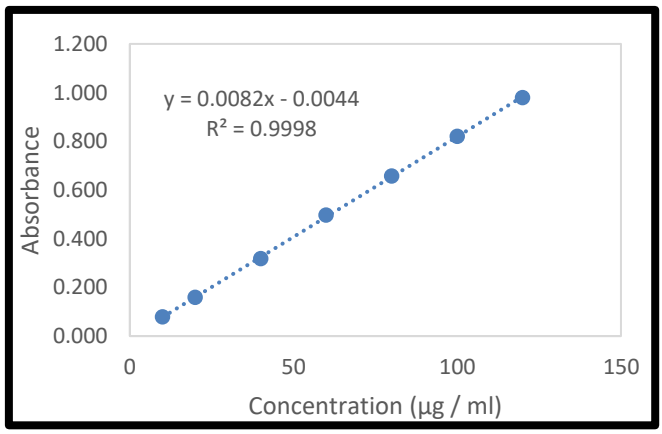

Figure 4: Calibration Curve of ROSU (10-120 µg/ml) at 310 nm in Distilled Water

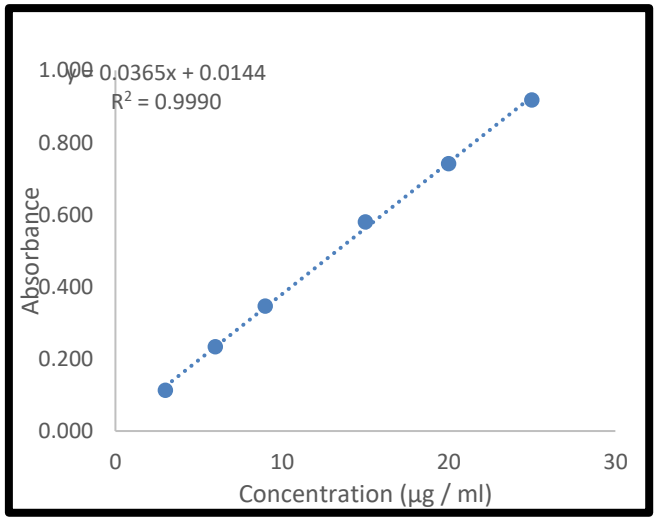

Figure 5: Calibration Curve of ROSU (3-25 µg/ml) at 243 nm in Distilled Water

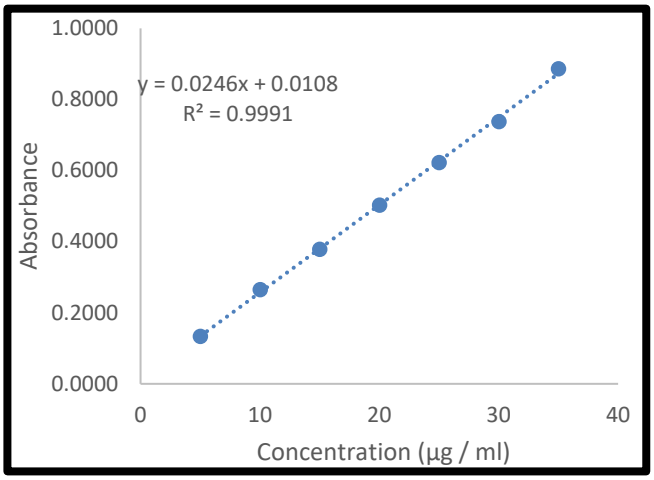

Figure 6: Calibration Curve of TENA (5-35 µg/ml) at 243 nm in Distilled Water

Table 1: Linear Regression analysis for ROSU and TENA

Drugs Parameters	ROSU		TENA
Wavelength (nm)	310	243	243
Beer's law limit (µg/ml)	10 to 120	3 to 25	5 to 35
Correlation Coefficient (R^2)	0.9998	0.9990	0.9991
Slope	0.0082	0.0365	0.0246
Intercept	0.0044	0.0144	0.0108
A1%1cm	82	365	246
LOD (µg/ml)	1.095	0.243	0.535
LOQ (µg/ml)	3.320	0.735	1.622

In this method, two wavelengths 243 nm (λ1) and 310 nm (λ2) were selected in such a way that absorbance of TENA is nearly zero at the analytical wavelength of ROSU (310 nm). The concentrations of two drugs in mixture were calculated by using following equations:

$$C_{ROSU} = A_1/ax_1$$

$$C_{TENA} = (A_2 - ax_2 C_{ROSU})/ay_1$$

Where, C_{ROSU} and C_{TENA} are the concentrations of ROSU and TENA respectively. A1 and A2 are the absorbances of the mixture at 310 nm and 243 nm respectively. ax_1 and ax_2 are the Absorptivities of ROSU at 310 nm and 243 nm respectively; whereas, ay_1 is the Absorptivity of TENA at 243 nm.

Analysis of Tablet Formulation

Twenty Tablets of ROSU and TENA (Cedaglip - R10, Chemo Biological) (ROSU-10 mg and TENA-20 mg) were weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 10 mg of ROSU (20 mg of TENA) was transferred to 100 ml volumetric flask and sonicated for 20 minutes with 70 ml of distilled water for extraction of drugs and the volume was made up with distilled water. The resulting solution was filtered through Whatman filter paper no. 41, few ml of filterate was discarded, and rest of solution was used as 'Sample Stock A'. (~100 µg/ml of ROSU, 200 µg/ml of TENA)

From this stock solution, 1ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask and the volume was made up to the mark with distilled water (~10 µg/ml of ROSU and 20 µg/ml of TENA). The absorbance of above solution was measured at 310 nm and 243 nm in order to calculate assay. The % Assay was calculated using A1%1cm values of ROSU and TENA. The results are shown in Table 2.

Table 2: Results of Marketed Formulation

Analyte	Label Claim (mg/tab)	% Label Claim Estimated (Mean ± SD)	% RSD
ROSU	10	99.59 ± 0.9928	0.9969
TENA	20	100.21 ± 1.0241	1.0219

Method Validation

The proposed method was validated in accordance to ICH guidelines[23]. Accuracy of the method was determined using recovery study by standard addition method at three different levels 80%, 100% and 120% of assay concentration and percentage recovery were calculated. Precision of the method was determined using Intermediate precision: Intra-day precision, Inter-day precision, Variation by different analyst and Repeatability study. LOD and LOQ was determined by following formula

$$LOD = 3.3 \times \frac{\text{Standard Deviation of y–Intercepts of Six Calibration curves}}{\text{Average Slope of Six Calibration Curves}}$$

$$LOQ = 10 \times \frac{\textit{Standard Deviation of y–Intercepts of Six Calibration curves}}{\textit{Average Slope of Six Calibration Curves}}$$

RESULTS AND DISCUSSION

To develop absorbance correction method for simultaneous estimation of ROSU and TENA, the drug solutions were prepared in suitable concentration (10 µg/ml of ROSU and 20 µg/ml of TENA) in distilled water. These solutions were scanned from 200-400 nm and overlay spectra was observed for selection of analytical wavelength. It was observed that at 310 nm ROSU was showing good absorbance where TENA is showing zero absorbance. So, 310 nm was selected as an analytical wavelength for ROSU. The concentration of ROSU was determined as a single drug estimation using A1% 1 cm value as 82. To determine ROSU absorbance at 243 nm, A=abc equation was used and absorbance of ROSU at 243 nm was calculated using A1% 1cm value as 365. From the total absorbance at 243 nm absorbance due to ROSU was nullified to get absorbance because of TENA alone. Further TENA concentration was determined using A1% 1cm value as 246 at 243 nm.

Using this method, the absorbance of marketed formulation (CEDAGLIP R-10) (ROSU-10 mg and TENA-20

mg) was measured at 243 nm and 310 nm and amount of drugs present were found using A1%1cm values of the respected drugs at their analytical wavelength are given in Table 4**.** Percentage assay of ROSU and TENA were found as 99.59 % and 100.21 % respectively.

The developed absorbance correction method for simultaneous estimation of ROSU and TENA was validated as per ICH guidelines and was found linear in the concentration range of 10-120 µg/ml for ROSU with R^2 value as 0.9998 at 310 nm and that for TENA the method was linear in the concentration range of 5-35 µg/ml with R^2 value as 0.9991 at 243 nm. As the R^2value is more than 0.999 the method showed satisfactory linearity in the selected range. Accuracy was determined by standard addition method (80%, 100% and 120% of the assay level), % recoveries of ROSU and TENA were found to be in the range of 98.07-101.12 % and 99.07-100.43 % respectively. Precision of the method was determined using intra-day precision, inter-day precision and repeatability study. It was found that %RSD for all these studies and both the drug were less than 2%. Variation of the results using two different analytes was determined by Performing assay (n=3). As calculated values of F-test and t-test were found less than the tabulated values hence no significant difference was observed between the results of two analysts. LOD and LOQ of ROSU were found to be 1.095 and 3.320 µg/ml at 310 nm, respectively. LOD and LOQ value of TENA were found to be 0.535 and 1.622 µg/ml at 243 nm, respectively.

CONCLUSION

A simple, precise, and sensitive UV-spectrometric method utilizing the absorbance correction technique has been developed for the simultaneous estimation of Rosuvastatin and Teneligliptin. This eco-friendly method employs distilled water as the solvent, making it economically viable.

The developed method was rigorously validated according to ICH guidelines, and the results of the assay and validation studies were satisfactory. Therefore, this method can be successfully applied for routine analysis of Rosuvastatin and Teneligliptin in marketed formulations within the pharmaceutical industry.

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