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DEVELOPMENT AND VALIDATION OF FIRST-ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF GEMIGLIPTIN TARTRATE AND ROSUVASTATIN CALCIUM IN SYNTHETIC MIXTURE

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ABSTRACT

This work aimed to develop and validate a simple, accurate, precise, and reproducible spectroscopic method for simultaneous estimation of Rosuvastatin calcium and Gemigliptin tartrate by UV-Visible first-order derivative method. According to our present knowledge, no UV method was reported for combination of Rosuvastatin Calcium and Gemigliptin tartrate. So, in this work, it was decided to performed the first-order derivative method and it was validated as per ICH(Q2 R1) guideline. Rosuvastatin calcium and Gemigliptin tartrate showed absorbance at the working wavelength of 242nm (Zero crossing point of Rosuvastatin calcium) and 248nm (Zero crossing point of Gemigliptin tartrate) respectively, using methanol as diluent. The linearity of this method was found in the concentration range of 20 to 300 µg/ml for Gemigliptin Tartrate and Rosuvastatin Calcium. The correlation coefficient r value was found to be 0.9985 for Gemigliptin Tartrate and 0.998 for Rosuvastatin Calcium. The method was extensively validated according to ICH guidelines for Linearity, Range. Precision, specificity and Robustness.

KEYWORDS: UV, Validation, Gemigliptin Tartrate, Rosuvastatin Calcium.

INTRODUCTION

Zemiglo, whose generic name is Gemigliptin (rINN), is classified as an oral anti- hyperglycemic agent, belonging to the dipeptidyl peptidase-4 inhibitor (DPP-4 inhibitor) category of medications used in diabetes treatment.[1] The New Drug Application (NDA) for gemigliptin was presented to the Korea Food & Drug Administration (KFDA) in July 2011, receiving approval in June 2012. Rosuvastatin calcium(Fig. 1A), an new member of "statins" family competitively inhibits an enzyme called as HMG-CoA reductase which is an rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A into mevalonate, a precursor to cholesterol.[2-3] Chemically, Rosuvastatin calcium is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methyl methane sulfonamido)[[6-(propan-2-yl) pyrimidin-5-yl]3,5 dihydroxyheptenoic acid calcium salt.[4-5] A monolayer tablet containing a fixed-dose combination(FDC) of gemigliptin and rosuvastatin 50/20 mg is utilized for the treatment of patients dealing with type 2 diabetes mellitus and dyslipidemia.[6]

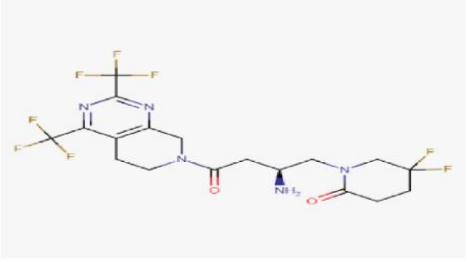

Figure: A

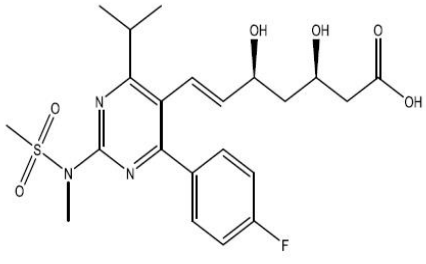

Figure: B

Figure 1: Chemical structure of [A] Gemigliptin tartrate [B] Rosuvastatin calcium.

Materials

Methanol and Acetonitrile and water of HPLC grade was procured from Rankem Mumbai India Pvt. Ltd. Potassium Dihydrogen Phosphate, Sodium Hydroxide, Triethyl amine were procured from Chemdyes Pvt Ltd.

Active pharmaceutical ingredients

Sample of Rosuvastatin (ROS) and Gemigliptin (GEM) procured from Sun Pharmaceutical Ltd, Gujarat.

Preparation of stock solution

The stock solution was prepared by accurately weighing and transferring approximately 20mg of Rosuvastatin Calcium (ROS) and 50 mg of Gemigliptin tartrate (GEM) into a 100 ml volumetric flask. Then, 50 ml of methanol was added, and the solution was sonicated to dissolve the compounds. The volume was adjusted to the mark with the diluent. This resulted in a concentration of 200 µg/ml for Rosuvastatin Calcium (ROS) and 500 µg/ml for Gemigliptin tartrate (GEM). Furthermore, 2 ml of the above solution was diluted into a 10 ml volumetric flask and the volume was made up to the mark with the diluent. This dilution resulted in a concentration of 40 µg/ml for Rosuvastatin Calcium (ROS) and 100 µg/ml for Gemigliptin tartrate (GEM).

Selection of wavelength

Accuately weighed 20 mg of Rosuvastatin (ROS) and 50mg of Gemigliptin (GEM) were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and then volume was adjusted to the mark with methanol to obtain concentration of 1000 µg/ml. These solutions were further diluted to obtain concentration of 200 µg/ml of Rosuvastatin (ROS) and 500 µg/ml of Gemigliptin (GEM). These standard solutions of were further diluted to obtain the 10 µg/ml of Rosuvastatin (ROS) and 25 µg/ml of Gemigliptin (GEM) in methanol were scanned in UV range, 200-400 nm in 1 cm cell using methanol as blank and maximum absorbance was measured for selection of λmax of both drugs.

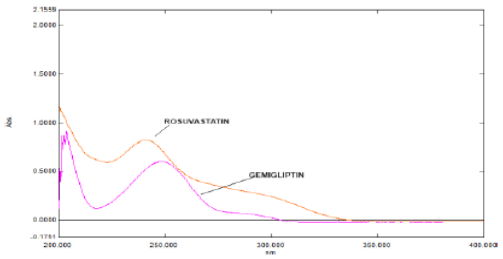

Figure 2: The overlain zero-order absorption spectra of Rosuvastatin Calcium (ROS) 20 µg/ml and Gemigliptin tartrate (GEM) 50 µg/ml.

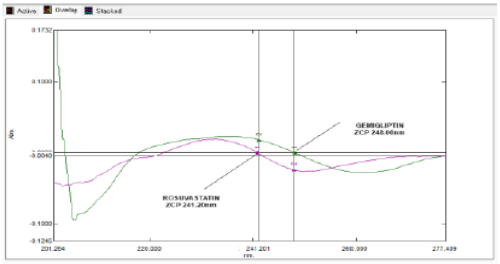

Figure 3: First-order derivative spectra with scaling factor = 2 and Δλ = 2 for Rosuvastatin Calcium (ROS) 20 µg/ml and Gemigliptin tartrate (GEM) 50 µg/ml.

RESULTS AND DISCUSSION

Method validation

The developed method was validated following ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ), robustness.

Optimization of UV Spectroscopy Condition

The derivative spectrophotometric method is one of the advanced modern spectrophotometric techniques that offer a useful means for extracting both qualitative and quantitative information from the spectra composed of overlapped bands. It is based on using the first- or higher-order derivatives of absorbance with respect to wavelength from parent zero-order ones. Because derivatization can lead to the separation of unresolved signals and reduction of spectral background interferences, this technique permits the quantification of one analyte in the presence of others without initial separation or purification. In First order derivative method, solutions of Rosuvastatin Calcium (ROS) 20 µg/ml and Gemigliptin tartrate (GEM) 50 µg/ml, were prepared separately from the dilution of standard stock solution with methanol and scanned the spectra from 200nm to 400 nm. The spectra were derivatized for first order. From the overlain spectra of these drugs, the wavelengths selected for quantification were 241.20 nm for Gemigliptin tartrate (GEM) (Zero cross for Rosuvastatin Calcium (ROS), 248.0 nm for Rosuvastatin Calcium (ROS) (Zero cross for Gemigliptin tartrate (GEM).The overlain zero-order absorption spectra of Rosuvastatin Calcium (ROS) 20 µg/ml and Gemigliptin tartrate (GEM) 50 µg/ml, were obtained [Figure 4]. These absorption spectra were converted to first-order derivative spectra by using the instrument mode. After observing the overlain first-order derivative spectra with scaling factor = 2 and Δλ = 2 for Rosuvastatin Calcium (ROS) 20 µg/ml and Gemigliptin tartrate (GEM) 50 µg/ml, [Figure 7.2], zero crossing points of drugs were selected for the analysis of other drugs.

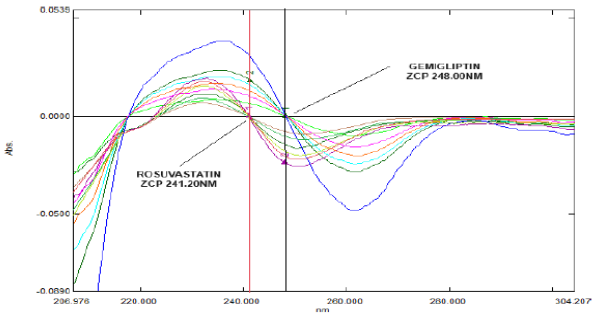

Figure 4: Overlay Chromatogram of First order of Rosuvastatin Calcium (ROS) and Gemigliptin tartrate (GEM).

Linearity

Linearity test solutions of ROS and GEM were prepared at concentration levels of 20 to 100 µg/ml and 50 to 250 µg/ml respectively. Linearity test solutions were prepared by diluting the stock solution to the required concentrations. Linearity was established by the least-squares linear regression analysis of the calibration data. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linearity curve for ROS and GEM was summarized in table no 1 and 2.

Gemigliptin tartrate (GEM), a calibration curve was constructed by plotting the mean peak area against concentrations ranging from 50 to 250 µg/ml. For Rosuvastatin Calcium (ROS), a separate calibration curve was generated by plotting the mean peak area against concentrations ranging from 20 to 100 µg/ml. The correlation coefficient (R^2) was determined to be 0.9985 for Gemigliptin tartrate (GEM) and 0.998 for Rosuvastatin Calcium (ROS). These high correlation coefficients indicate strong linear relationships between the concentration of the analytes and their respective peak areas. Both calibration curves exhibited linearity within their respective concentration ranges. The results of linearity are shown in Table 33 and Table 34 respectively. The overlay chromatogram of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) at 256 nm shows in Figure.

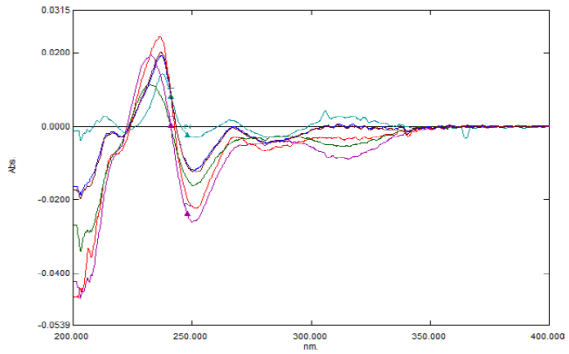

Figure 5: Overlay spectra of rosuvastatin calcium.

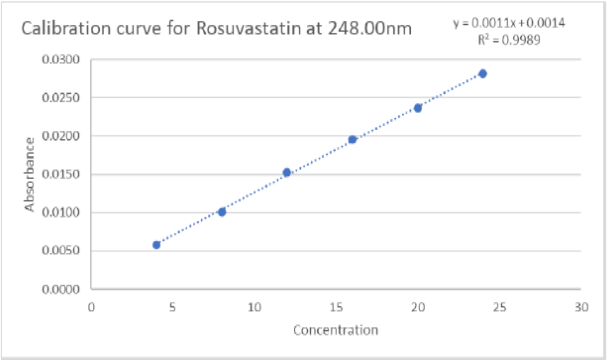

Figure 6: Calibration curve of rosuvastatin calcium.

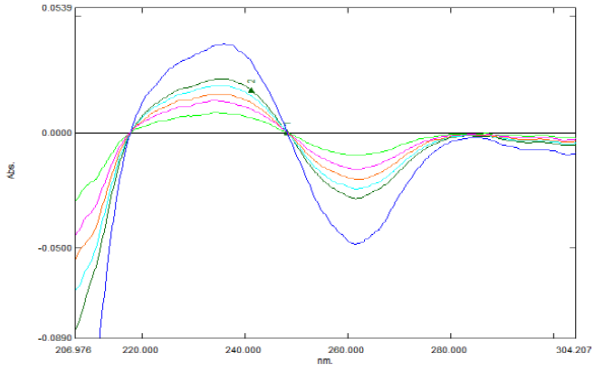

Figure 7: Overlay spectra of gemgigliptin tartrate.

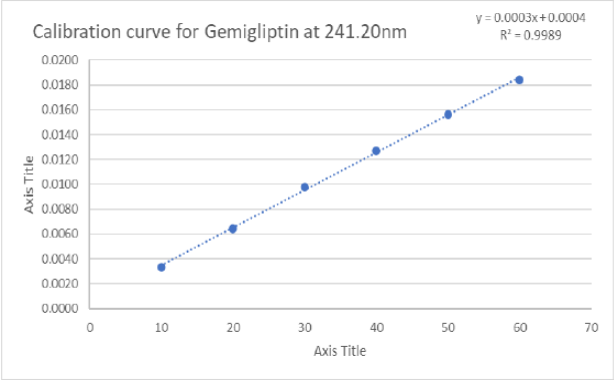

Figure 8: Calibration curve of gemigliptin tartrate.

Table 1: Calibration data of Rosuvastatin Calcium (ROS).

Concentration (µg/ml)	Absorbance Mean ± SD (n=6)	% RSD
4	0.0058±0.0001	1.72
8	0.0101±0.0002	1.98
12	0.0153±0.0003	1.96
16	0.0196±0.0002	1.02
20	0.0237±0.0004	1.69
24	0.0282±0.0005	1.77

Table 2: Calibration data of Gemigliptin tartrate (GEM).

Concentration (µg/ml)	Absorbance Mean ± SD (n=6)	% RSD
10	0.0033±0.00005	1.52
20	0.0064±0.00011	1.72
30	0.0098±0.00017	1.73
40	0.0127±0.00014	1.10
50	0.0156±0.0003	1.92
60	0.0184±0.00024	1.30

Accuracy

Accuracy of the method was confirmed by recovery study from synthetic mixture at three levels 50%, 100% and 150% of standard addition. The data shown in Table 3 indicate that the developed method is accurate. The% recovery of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) was found to be in range of 101.33 – 102.00% and 98.73 – 101.28 %, respectively.

Table 3: Accuracy data of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS).

Drug	% Level of spike	Amount of drug in sample (µg/ml)	Amount of std. added (µg/ml)	Total amount of drug (µg/ml)	Total amount of std. found (µg) Mean ± SD (n=3)	% Recovery
GEM	0	20	0	20	20.28 ± 0.43	101.40
	50	20	10	30	30.60 ± 0.21	102.00
	100	20	20	40	40.53 ± 0.15	101.33
	150	20	30	50	50.99 ± 0.21	101.98
ROS	0	8	0	8	7.86 ± 0.05	98.25
	50	8	4	12	12.18 ± 0.43	101.50
	100	8	8	16	16.25 ± 0.11	101.56
	150	8	12	20	20.27 ± 0.22	101.35

Repeatability

In RP-HPLC method, repeatability has been carried out by Injection repeatability. Injection repeatability was carried out by analyzing the sample solution of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) (150 + 60 µg/ml) six times and peak area was measured and % RSD which is shown in Table 4.

Table 4: Repeatability data of Gemigliptin (GEM) and Rosuvastatin Calcium (ROS)

Sr. No.	Gemigliptin (GEM) (150 µg/ml) Absorbance	Rosuvastatin Calcium (ROS) 60 µg/ml) Absorbance
1	0.00999	0.0156
2	0.00999	0.0156
3	0.00998	0.0156
4	0.00997	0.0153
5	0.00996	0.0154
6	0.00999	0.0155
Mean	0.00998	0.0155
SD	0.0001	0.0001
% RSD	1.00	0.65

Precision

Intraday and Inter day precision

The precision of the method was evaluated through both Intraday and Interday precision analyses. Sample solutions containing Rosuvastatin Calcium (ROS) 4, 12, 24 µg/mL and Gemigliptin (GEM) 10, 30, 60 µg/mL were analyzed at three different time intervals within a single day and on three different days, respectively. The % RSD (Relative Standard Deviation) was determined for each concentration level. The % RSD values were found to be less than 2%, indicating that the method exhibits good precision. Mean peak areas and % RSD values for each concentration level are provided in Table 5.

Table 5: Precision data of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS)

Drug	Conc. (µg/ml)	Intraday Precision		Inter day Precision	
		Area (Mean ± SD) (n=3)	% RSD	Area (Mean ± SD) (n=3)	% RSD
GEM	10	0.0034 ± 0.0001	1.84	0.0033 ± 0.0001	1.93
	30	0.0098 ± 0.0001	1.02	0.01 ± 0.0001	1.00
	60	0.0182 ± 0.0002	1.10	0.0185 ± 0.0003	1.62
ROS	4	0.0058 ± 0.0001	1.72	0.0059 ± 0.0001	1.69
	12	0.0154 ± 0.0001	0.65	0.0155 ± 0.0002	1.29
	24	0.0282 ± 0.0002	0.71	0.0287 ± 0.0005	1.74

Limit of Detection and Limit of Quantification

According to ICH, the approach based on the standard deviation of the response and mean of slope was used to determining the Limit of detection (LOD) and Limit of quantification (LOQ). The LOD and LOQ were found to be 1.08 and 3.27 µg/ml for GEM as well as 0.82 and 2.5 µg/ml for ROS, respectively indicating high sensitivity of the method in table 6.

Table 6: LOD and LOQ data.

Parameter	GEM	ROS
LOD	1.08	0.82
LOQ	3.27	2.5

Analysis of synthetic mixture

The developed and validated first order derivative UV spectrophotometry method was applied for determination of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) in synthetic mixture. The sample was analysed

three times. The data of Assay of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) in synthetic mixture is shown in Table 40. The % assay was found to be 102.77 % and 102.79 % for Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS), respectively.

Table 7: Data of determination of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) in synthetic mixture.

Drug	Conc. (µg/ml)	Amount found (µg/ml)	Absorbance	% Assay Mean ± SD (n=3)
Gemigliptin tartrate (GEM)	40	40.90	0.0127	102.77 ± 0.52
		41.12	0.0127	
		41.32	0.0128	
Rosuvastatin Calcium (ROS)	16	16.35	0.0194	102.79 ± 0.54
		16.49	0.0195	
		16.51	0.0196	

n= Average of Three determination

Summary of validation

Table 8: Summary of validation parameter for Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) by UV method.

Sr. no	Parameters	Result	
		Gemigliptin tartrate (GEM)	Rosuvastatin Calcium (ROS)
1.	Zero Crossing Point	248.00 nm	241.20 nm
2.	Concentration range (µg/ml)	10-60 µg/ml	4-24 µg/ml
3.	Regression coefficient (r2)	0.9989	0.9989
4.	Detection limit (µg/ml)	1.08	0.82
5.	Quantification limit (µg/ml)	3.27	2.5
6.	Accuracy (% Recovery) (n=3)	101.33 – 102.00%	98.73 – 101.28 %,
7.	Precision (% RSD)		
	Repeatability (n=6)	1.00	0.65
	Intraday Precision (n=3)	1.02-1.84	0.65 – 1.72
	intraday Precision (n=3)	1.00 – 1.93	1.29 -1.74
8.	Robustness	Robust	Robust

CONCLUSION

Simple, accurate, and reproducible spectrophotometric methods have been developed and validated for simultaneous estimation of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) in combined tablet dosage form. The first method is based on first-order derivative spectroscopy. From the overlain spectra of these drugs, the wavelengths selected for quantification were 241.20 nm for Gemigliptin tartrate (GEM) (Zero cross for Rosuvastatin Calcium (ROS), 248.0 nm for Rosuvastatin Calcium (ROS) (Zero cross for Gemigliptin tartrate (GEM)). For Gemigliptin tartrate (GEM), a calibration curve was constructed by plotting the mean peak area against concentrations ranging from 50 to 250 µg/ml. For Rosuvastatin Calcium (ROS), a separate calibration curve was generated by plotting the mean peak area against concentrations ranging from 20 to 100 µg/ml. The correlation coefficient (R^2) was determined to be 0.9985 for Gemigliptin tartrate (GEM) and 0.998 for Rosuvastatin Calcium (ROS). Mean recoveries for all three methods were found satisfactory. The % recovery of Gemigliptin tartrate (GEM) and Rosuvastatin Calcium (ROS) was found to be in range of 101.33 – 102.00% and 98.73 – 101.28 %, respectively. All methods were validated according to International Conference on Harmonization Q2B guidelines. The developed methods are simple, precise, rugged, and economical. The utility of methods has been demonstrated by analysis of both drugs in bulk and synthetic mixture.

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