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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ROSUVASTATIN AND EZETIMIBE IN CAPSULE DOSAGE FORM.

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ABSTRACT

Rosuvastatin and Ezetimibe are used for the treatment of hyperlipidemia. A stability-indicating RP-HPLC method was developed and validated for the estimation of Rosuvastatin and Ezetimibe in Capsule dosage form using Inertsil ODS 3 (100 mm x 4.6 mm i.d, 3 µm particle size) column with mobile phase consisting of Buffer solution (dissolve about 3.4 gm of Disodium hydrogen phosphate dihydrate in 1000 ml water and mix. Adjust pH of 5.0 with dilute phosphoric acid.) and acetonitrile (60:40 %v/v) with a flow rate of 1.5 ml/min (UV detection 232 nm). Linearity was observed over the concentration range 5.0-20 µg/ml for both Rosuvastatin (r2=0.9994) and Ezetimibe (r2=0.9990). The LOD and LOQ were found to be 0.0282 µg/ml and 0.0853 µg/ml for Rosuvastatin and the LOD and LOQ for Ezetimibe were 0.0297 µg/ml and 0.0901 µg/ml respectively. Rosuvastatin and Ezetimibe were subjected to stress conditions of degradation in aqueous solutions including acidic, alkaline, oxidation, Humidity and thermal degradation. The method was validated as per ICH guidelines. The percentage RSD for precision was found to be 0.4 % and 0.7 % for Rosuvastatin and Ezetimibe. The method is simple, specific, precise, robust and accurate for estimation of Rosuvastatin and Ezetimibe in Capsule dosage form. The method was successfully applied to the determination of these drugs in pharmaceutical dosage form.

KEYWORDS: Rosuvastatin, Ezetimibe, RP-HPLC, ICH guidelines, Stability Indicating, Validation.

INTRODUCTION

Rosuvastatin (RST) is chemically designated as (3R, 5S, 6E) - 7 - [4 - (4 - fluorophenyl) - 2 - (N – methyl methane sulfonamido) - 6 - (propane - 2 - yl) pyrimidin - 5 - yl] - 3, 5 - dihydroxyhept - 6 - enoic acid. It is a member of the drug class of statins. It is used in the treatment of Hyperlipidemia(Figure-1). Rosuvastatin Calcium is a selective and competitive inhibitor of hydroxyl methyl glutaryl coenzyme A (HMG Co A) reductase (a precursor of cholesterol), the rate- limiting enzyme that converts 3-hydroxyl-3-methylglutaryl coenzyme A to mevalonate. 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 (Figure-2). It is a selective cholesterol absorption inhibitor, used for the treatment of hyperlipidemia, which potentially inhibits the absorption of biliary and dietary cholesterol. Ezetimibe prevents intestinal absorption of cholesterol without affecting absorption of triglycerides, fatty acids and fat-soluble vitamins.

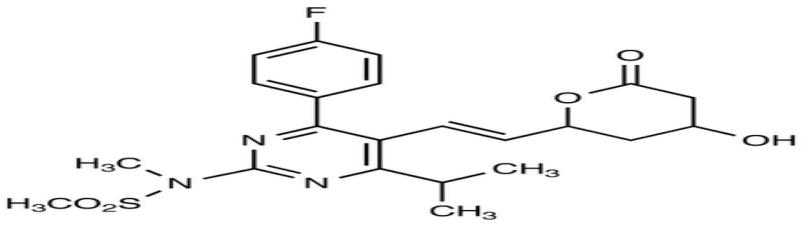

Figure. 1. Rosuvastatin Chemical structure.

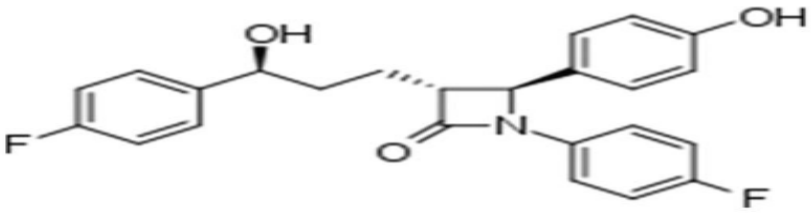

Figure. 2. Ezetimibe Chemical Structure.

Various analytical techniques such as micellar liquid chromatography, HPLC, HPTLC, densitometry TLC, spectrophotometry and spectrofluorimetry have been developed for the estimation of rosuvastatin and ezetimibe in pharmaceutical formulations. In the present study an attempt has been made to develop a validated stability indicating RP-HPLC method for the estimation of rosuvastatin and ezetimibe in pharmaceutical formulations as per ICH guidelines.

MATERIALS AND METHODS

Apparatus

RP-HPLC instrument equipped with an UV –Visible detector and a photodiode array detector (ultimate dionex 3000 , Shimadzu Japan, Waters) ,an auto-Sampler ,Uv –visible spectroscopy (Shimadzu-1700 Series, Japan) ,Analytical Balance (Mettler Toledo, India) , A Hot air oven (Grover, New Delhi) , Digital pH meter (Lab India , Pico+ , Mumbai) ,Sonicator (Ultrasonic cleaner ,Frontline FS 4, Mumbai), Milli Q water System (Siemens Ultra clear,Malaysia), Water Bath (Equitron , Mumbai), Pre-validated volumetric flasks, pipettes, etc.

Chemicals and Reagents

Rosuvastatin and Ezetimibe standard powder were gifted by Zydus Cadila Healthcare Ltd.
The capsule formulations containing 10 mg rosuvastatin and 10 mg ezetimibe were procured From Zydus Cadila Healthcare ltd. Methanol and Acetonitrile, Water
(HPLC & Spectroscopy grade, Spectrochem pvt ltd, Mumbai)
Hydrochloric acid, Sodium Hydroxide and Disodium Hydrogen Phosphate Dihydrate Salt
(L R grade, Merck Pharmaceuticals Ltd, India)

Chromatographic Conditions

The chromatographic separations were performed using Inertsil ODS 3 (100 mm x 4.6 mm i.d, 3 µm particle size) column at 25 ºC temperature. The optimum mobile phase consisted of Buffer solution (dissolve about 3.4 gm of Disodium hydrogen phosphate dihydrate in 1000 ml water and mix. Adjust pH of 5.0 with dilute phosphoric acid.) and acetonitrile in the ratio of 60:40 %v/v. Auto sampler 30 µl was used and kept at Room temperature. Analysis was done with flow rate of 1.5 ml/min at 232 nm (λ max of Rosuvastatin and Ezetimibe) wavelength by using photodiode array (PDA) detector.

Software

Chromeleon software Ultimate Dionex 3000 HPLC.

Preparation of standard stock solution of Rosuvastatin

Transfer an accurately weighed quantity of about 52 mg of Rosuvastatin Calcium and add into a 100 ml volumetric flask, add about 50.0 ml of diluent & sonicate to dissolve. Make volume up to the mark with diluent and mix it.

Preparation of standard stock solution of Ezetimibe

Transfer an accurately weighed quantity of about 50 mg of Ezetimibe and add into a 100 ml volumetric flask, add about 50.0 ml of acetonitrile & sonicate to dissolve. Make volume up to the mark with acetonitrile and mix it.

Preparation of standard stock solution of Rosuvastatin and Ezetimibe

Dilute 2.0 ml of each Rosuvastatin standard stock and Ezetimibe standard stock Solution; add into a 100 ml volumetric flask and diluted up to the mark with Mobile phase & mix it.

Calibration curves of RSV and EZE

Calibration curve were plotted over a concentration range of 5-20 µg/ml for RSV and EZE drugs. For this aliquots of 5, 8, 10, 12, 15, 18, and 20 µl for RSV and EZE. The calibration curves were obtained by plotting peak area Vs concentration (µg/ml) of RSV and EZE the regression equation was calculated.

Determination of wavelength of maximum absorbance

The standard solution of RSV and ezetimibe was scanned into UV. It is detected between the range of 200-400 nm. Maximum absorbance was achieved at 232 nm; both drugs were detected at this wavelength.

Validation of the Developed Method

Linearity

A calibration curve was plotted in concentration range from 5-20 µg/ml for RSV and EZE 5-20 µg/ml was used. Linearity curve plotted by taking aliquots of 5,8,10,12,15,18 and 20 µg/ml for RSV and EZE. The solution was filtered and diluted with Diluent (Water: ACN). 30 µl were injected from this solution to HPLC. Calibration curve plotted by putting peak area vs. concentration on axis then regression line equation was calculate.

Precision

The repeatability of the instrument was checked by repeatedly solutions (n = 6) solution of RSV (10 µg/ml) and SMV (10 µg/ml). Without changing the parameters of the developed method It showed low % RSD of peak area of RSV and EZE. The inter-day and intra-day variation was determined at three different concentration levels on three different days over a period of one week (interday precision) and three different times on same day (intraday precision).

Limit of detection and limit of quantification

LOD and LOQ of the drug were calculated by using the following equations designated by ICH guideline: LOD = 3.3 X σ/S and LOQ = 10 X σ/S.

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Recovery studies

The accuracy of the method was determined by calculating recoveries of RSV and EZE by the standard addition method. Known amounts of standard solution of RSV and EZE were added at 50 %, 100 % and 150 % levels to pre quantified sample solutions of RSV and EZE. Accuracy was determined in terms of percentage recovery. The experiment was conducted in triplicate.

Specificity

The specificity of the method was ascertained by analyzing blank sample solution and sample solution of RSV and EZE. The peak purity was assessed for blank, standard solution and sample solution for both the drugs.

System suitability: In this system suitability method, standard drug solutions of RSV and EZE were injected for six times to check suitability for instrument. The results were calculated in % RSD which isn't more than 2.

Solution stability: The solution stability of Rosuvastatin and Ezetimibe in the assay method was carried out by leaving both the sample and reference standard solutions in tightly capped volumetric flasks at room temperature for 20 h. The same sample solutions were assayed at 3 hrs intervals over the study period.

Filter Compatibility: In this method, drug sample solutions of Rosuvastatin and ezetimibe were filtered with 0.45 PVDF Filter and 3ml, 5ml, 7 ml discarded samples were compared with unfiltered sample (initial) & Calculate % Difference between filtrate and unfiltrate.

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (232 and 230 nm, 234nm), column temperature (20, 25,30C) and flow rate (1.4 and 1.5, 1.7 ml/min). Robustness of the method was studied using three replicates at a concentration level of 10 µg/ml of Rosuvastatin and Ezetimibe.

Assay of RSV AND EZE

Weigh 10 tablets and calculate average weight. Weigh and transfer 5 intact tablets in to 200.0 ml volumetric flask. Add about 10 ml of water and sonicate till completely disperse The tablets. And add about 125 ml of Diluent and sonicate for 30 minutes with occasional Shaking. Make volume up to the mark with diluent and mix. Filter the solution through 0.45 µm Millipore PVDF filter, collect the filtrate by discarding first 5.0 ml of the filtrate And Dilute 4.0 ml of the filtrate to 100.0 ml with mobile Phase and mix.

Forced degradation studies

Stress studies were performed to evaluate the specificity of the method. All samples were diluted with mobile phase to give a final concentration 10 µg/ml and filtered through 0.45 µm PVDF filter before inject it.

Acidic conditions

Acidic degradation was conducted by dissolving 1 intact tablet into 50 ml volumetric flask and add 2.5 ml water to sonicate till completely disperse the tablet, & add almost 1 ml 0.01 N of Hcl and Add into the volumetric flask , reflux at 60℃ for 30 min in reflux bath. And after all the process, neutralized with 1 ml 0.01 N NaOH and mark with diluent. Lastly, dilute 1 ml into the 20 ml volumetric flask. 30µl solution was injected into the HPLC system.

Alkaline conditions

Alkaline degradation was conducted by dissolving 1 intact tablet into 50 ml volumetric flask and add 2.5 ml water to sonicate till completely disperse the tablet, & add almost 1 ml of 0.01 N NaOH and Add into the volumetric flask, reflux at 40℃ for 10 min in reflux bath for RSV and at 60℃ for 30 min in reflux bath for EZE. And after all the process, neutralized with 1 ml of 0.01 N Hcl and mark with diluent. Lastly, dilute 1 ml into the 20 ml volumetric flask. 30µl solution was injected into the HPLC system.

Oxidation conditions

Oxidation degradation was performed by treating the drug solution mixture (containing 1intact tablet of RSV and EZE) and add 1 ml of 10% H_2O_2, reflux at 60℃ for 10 min. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement and 30 µl of the solution was injected in to the HPLC system.

Neutral conditions: Neutral degradation was performed by treating the drug solution mixture (containing 1intact tablet of RSV and EZE) with 1 ml of water at Room temperature for 24 hrs. And Make up with mobile phase as per requirement and 30 µl of the solution was injected in to the HPLC system.

Humidity conditions: Humidity degradation was performed by treating the drug solution mixture (containing 1intact tablet of RSV and EZE) in Humidity chamber maintained at 50℃, 80% for 24 hrs and cooled, And Make up with mobile phase as per requirement and 30 µl of the solution was injected in to the HPLC system.

Thermal conditions

The drug solution mixture (containing 1 intact tablet of RSV and EZE) was in a thermostat maintained at 105ºC for 24 hours, cooled and 30 µl of the solution was injected in to the HPLC system after necessary dilution with mobile phase.

RESULTS AND DISCUSSION

Method Development: In this work, a method based on RP-HPLC, using PDA detector, was developed and validated for Rosuvastatin and Ezetimibe in Pharmaceutical formulations. The experimental conditions were selected after different stationary and mobile phases were tested. C8 and C18 columns were used. However, best results were observed when the Inertsil ODS 3 (100 mm x 4.6 mm i.d, 3 µm particle size) column at 25 ºC temperature was used. The mobile phase, Buffer solution (dissolve about 3.4 gm of Disodium hydrogen phosphate dihydrate in 1000 ml with water and mix. Adjust pH of 5.0 with dilute phosphoric acid.) and acetonitrile in the ratio of 60:40 % v/v was used. The retention Time was found to be 3.03 min for Rosuvastatin and 11.89 min for ezetimibe. The duration of separation of two drugs are 7 min. so, It is superior method.

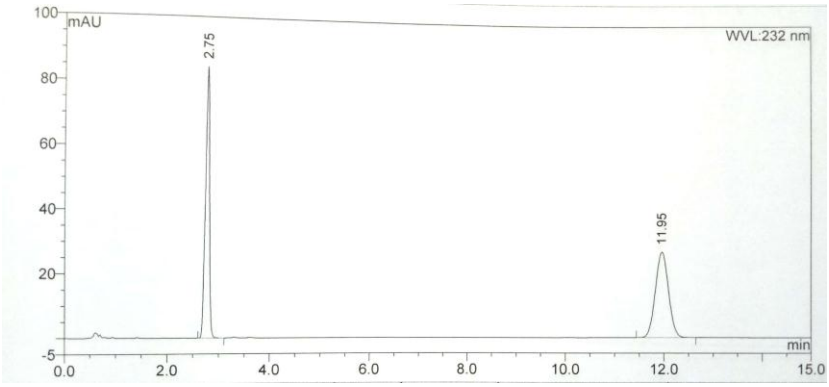

Figure. 3. Method Development studies of RSV and EZE.

Validation data of the method

Linearity

The combination of RSV and EZE shows linearity over a concentration range of 5.0-20 µg/ml (Table) and the linear regression equations were found to be y = 3832.2x – 5026.07 (r2=0.9994) and y = 4697.1x + 4099.7 (r2=0.9990) for Rosuvastatin and Ezetimibe respectively. (This is shown in Table. I).

Table. I summary of linear regression analysis and optical characteristics of RSV & EZE

Parameter	RSV	EZE
Wavelength , nm	242 nm	232 nm
Linear concentration range, µg/ml	5-20 µg/ml	5-20 µg/ml
Regression equation	y = 3832.2x – 5026.07	y = 4697.1x + 4099.7
Correlation coefficients, r	r2=0.9994	r2=0.9990
LOD, µg ml-1	0.0282 µg/ml	0.0853 µg/ml
LOQ, µg ml-1	0.0297 µg/ml	0.0901 µg/ml

Recovery studies

The accuracy study was repeated over three consecutive days and the resultant % RSD was found to be 0.2-0.3 and 0.1-0.6 for Rosuvastatin and Ezetimibe indicating that the method is precise. The recovery of for Rosuvastatin and Ezetimibe was found to be 99.3- 101.9 % and 102.8-103.6 % respectively (Table.II).

Table. II recovery studies of RSV & EZE.

Parameter	Level	Mg Added	Mg Recovered	% Recovery	Mean % Recovery	% RSD
Rosuvastatin	50 %	25.0	24.86	101.6	101.9	0.3
	100 %	25.0	49.15	100.4	100.5	0.2
	150 %	25.0	73.07	99.5	99.3	0.2
Ezetimibe	50 %	25.0	25.07	102.4	103.0	0.6
	100 %	25.0	50.34	102.8	102.8	0.1
	150 %	25.0	76.15	103.7	103.6	0.1

Precision

The % RSD for precision was found to be 0.4 and 0.7 for Rosuvastatin and Ezetimibe respectively.(Table.III)

Table. III Precision studies of Rosuvastatin and ezetimibe.

Precision	Label Claim Of RSV	Label claim Of EZE	Mean Assay % Of RSV	Mean Assay % OF EZE	% RSD Of RSV	% RSD Of EZE
Set-I	10.29	9.63	102.5	95.7	0.4	0.7
Set-II	10.26	9.57				
Set-III	10.31	9.66				
Set-IV	10.23	9.54				
Set-V	10.20	9.52				
Set-VI	10.21	9.50				

Limit of quantification (LOQ) and limit of detection (LOD)

The LOD and LOQ were found to be 0.0282 µg/ml and 0.0853 µg/ml for Rosuvastatin and the LOD and LOQ for Ezetimibe were 0.0297 µg/ml and 0.0901 µg/ml respectively.

System suitability

Standard response of Rosuvastatin and ezetimibe were found to be 0.2 % and 0.1 % RSD respectively. System is specific and precise.

Solution stability

The Sample solution of Rosuvastatin and Ezetimibe were stable for 16 hrs to 24 hrs time period. Difference between the sample solution was according to 0.6 to 0.9 % and 0.1 to 0.4 % for Rosuvastatin and ezetimibe respectively. (Table.IV)

Table. IV. Solution Stability studies of Rosuvastatin & Ezetimibe.

Parameter	Time (Hrs.)	Area of RSV	Area Of EZE	% Difference Of RSV	% Difference Of EZE
	Initial	465.205	509.020	-	-
	3	467.833	509.672	0.6	0.1
	6	468.039	510.168	0.6	0.2
	9	468.751	510.685	0.8	0.3
	12	469.400	511.044	0.9	0.4
	16	469.510	511.233	0.9	0.4

Filter Compatibility

Sample solution of Rosuvastatin and ezetimibe were filtered through the 0.45 PVDF filter and difference between the sample solution of Rosuvastatin and ezetimibe were accordingly -0.1 to -0.8 % and -0.3 to -1.0 % , It was pass for 0.45 PVDF Filter.(Table.V)

Table. V Filter Compatibility of Rosuvastatin and Ezetimibe

Parameter	Time (Hrs.)	Area of RSV	Area Of EZE	% Difference Of RSV	% Difference Of EZE
	Initial	471.231	512.250	-	-
	3 ml	470.975	510.933	-0.1	-0.3
	5 ml	468.937	507.194	-0.5	-1.0
	7 ml	467.255	506.947	-0.8	-1.0

Robustness: Usually a slight change in flow rate, Column temperature, etc. affects the chromatographic response such as retention time, tailing factor and theoretical plates etc. During this study the Theoretical plates were found to be more than 2000 for both the drugs and at the same time the % RSD was found to be < 2.0% (0.1-0.6% and 0.2-0.9 % for Rosuvastatin and Ezetimibe respectively) indicating that the proposed method is robust. The results were shown in Table.VI.

Table. VI Robustness studies of Rosuvastatin & Ezetimibe.

Parameter		Mean Area of RSV	Mean Area of EZE	% RSD OF RSV	% RSD OF EZE
Column Temperature	CT 20 C	389.915	475.571	0.4	0.7
	CT 25 C	386.247	466.344	0.1	0.2
	CT 30 C	390.154	477.225	0.4	0.8
Flow Rate	Flow Rate 1.4	391.729	479.725	0.5	0.4
	Flow Rate 1.5	386.247	466.344	0.1	0.2
	Flow Rate 1.7	340.864	421.123	0.3	0.3
Wavelength	WL-230	390.556	480.548	0.4	0.4
	WL-232	386.247	466.344	0.1	0.2
	WL-234	388.696	485.390	0.6	0.9

Assay of RSV AND EZE

The proposed method was applied for the determination of Rosuvastatin and Ezetimibe. And The % Assay was found 102% and 98.9 % for Rosuvastatin and ezetimibe.(Table.VII)

Table. VII Assay of Rosuvastatin and Ezetimibe.

Formulations	Label claim (mg)		AmountFound (mg)		Assay (%)	
	RSV	EZE	RSV	EZE	RSV	EZE
Formulation-I	10	10	10.20	9.89	102.0 %	98.9 %
Formulation-II	10	10	10.20	9.87	102.0 %	98.7 %

Forced degradation studies: Rosuvastatin is highly resistant towards acidic, alkaline, oxidation, neutral, Humidity and thermal degradations as the percentage of degradation was found to be 15 to 20 % for Rosuvastatin and Ezetimibe in Acid Degradation. In alkali degradation, 8 % and 2 % for Rosuvastatin and ezetimibe respectively. Oxidation degradation

for RSV and EZE were found 6 to 5 %. And there were no degradation found in neutral studies. Thermal degradation was found to be 20 -18 % for RSV and EZE at 105℃ 24 Hrs. and also Humidity degradation was found to be 20 to 15% in Rosuvastatin and ezetimibe. The degradation studies and specificity results were shown in Table.VIII.

Table. VIII Forced degradation studies of Rosuvastatin & Ezetimibe.

Stress Conditions	% Recovered	% Decomposed	Purity Angle	Purity Threshold
Rosuvastatin				
Acidic Degradation	85.8	16.2	0.758	0.899
Alkaline Degradation	91.6	10.4	0.712	0.844
Oxidative Degradation	87.6	14.4	1.641	4.620
Neutral Degradation	101.2	-	0.691	0.925
Thermal Degradation	81.4	20.6	0.735	6.992
Humidity Degradation	88.1	13.9	0.575	0.674

Stress Conditions	% Recovered	% Decomposed	Purity Angle	Purity Threshold
Ezetimibe				
Acidic Degradation	80.6	17.4	2.416	4.022
Alkaline Degradation	87.7	10.3	1.011	2.526
Oxidative Degradation	92.8	5.2	40.837	90.000
Neutral Degradation	98.3	-	1.801	4.939
Thermal Degradation	84.5	13.5	0.755	17.140
Humidity Degradation	80.2	17.8	0.484	1.541

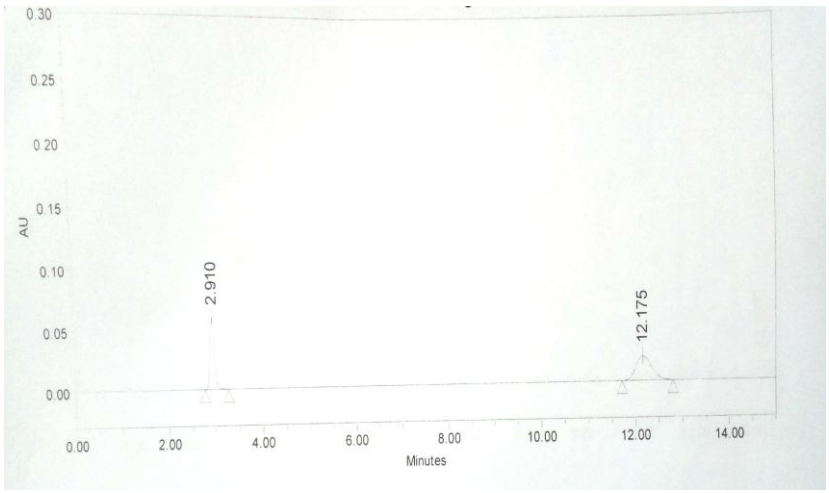

Figure. 4. Acid Degradation of RSV and EZE.

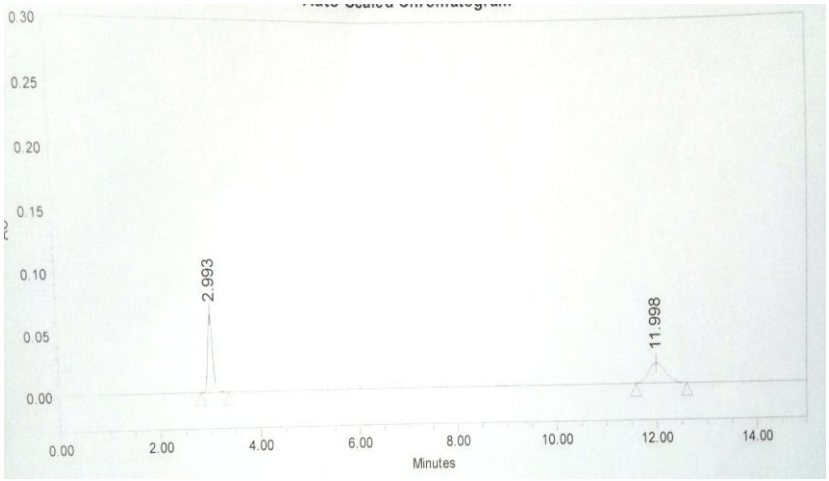

Figure. 5. Alkaline degradation of RSV and EZE.

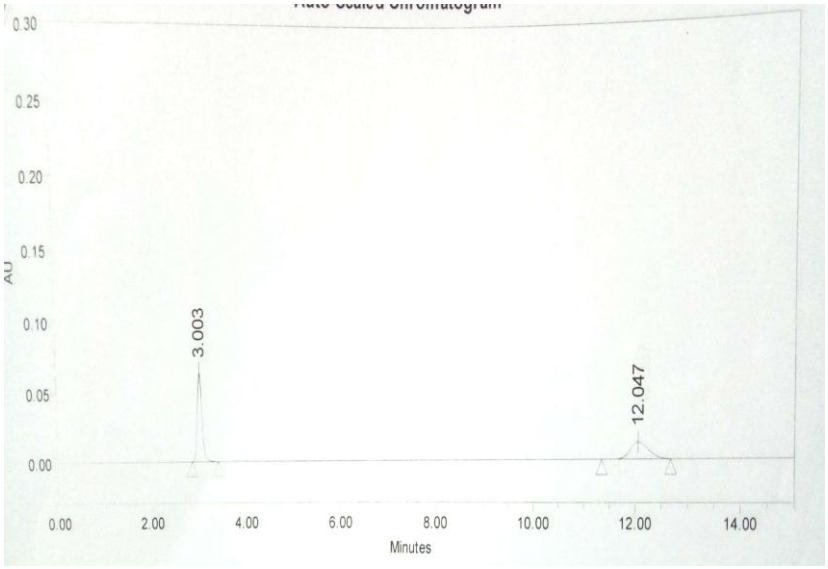

Figure. 6. Neutral degradation of Rosuvastatin and ezetimibe.

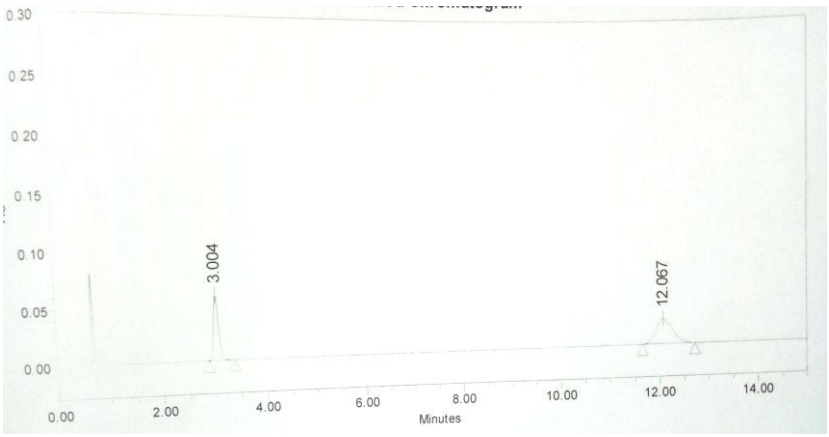

Figure. 7. Oxidative Degradation of RSV AND EZE.

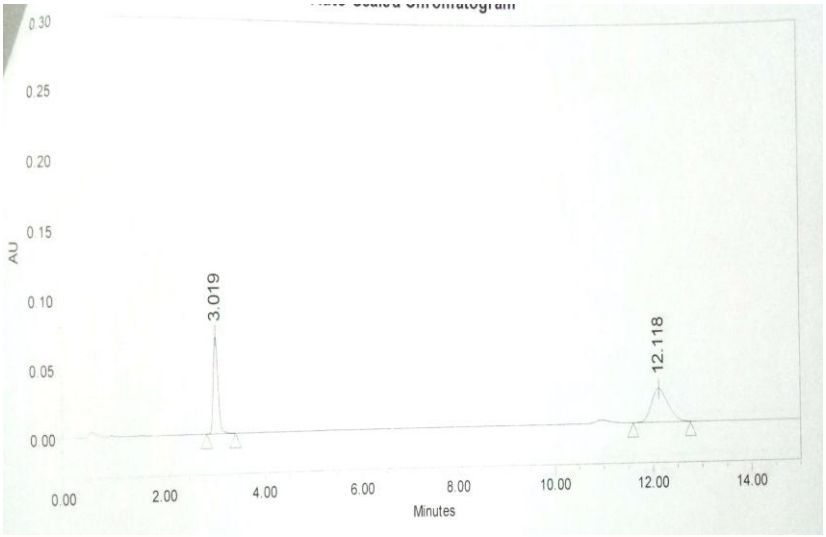

Figure. 8. Thermal degradation of Rosuvastatin and Ezetimibe.

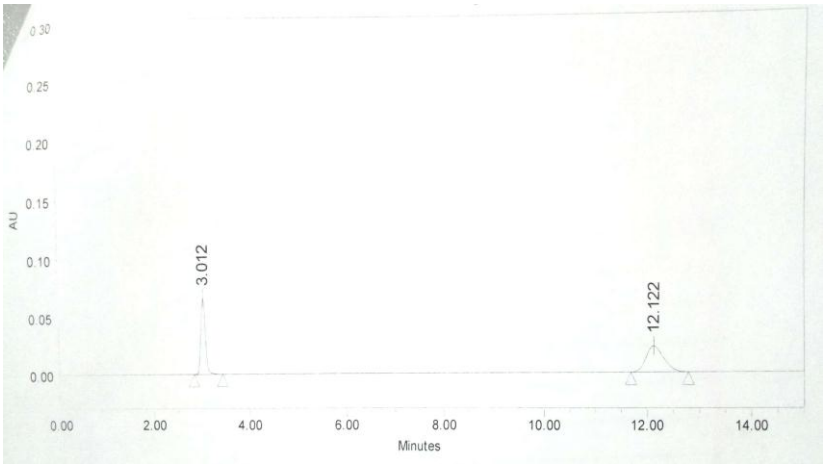

Figure. 9. Humidity degradation of RSV and EZE.

CONCLUSION

The High performance liquid chromatography method was developed for determination of RSV and EZE in Pharmaceutical formulations. Method was found to be precise and accurate as can be reflected from validation parameter data. Developed method was efficiently applied for determination of RSV and EZE in capsule formulations and there for method can be extended for the analysis of formulation.

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