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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND EZETIMIBE IN TABLET DOSAGE FORM

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ABSTRACT
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Simvastatin and Ezetimibe in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Symmetry C8 (4.6mm x 150mm, 5µm) column at ambient temperature with a mixture of ortho phosphoric acid buffer and Acetonitrile in the ratio 40:60 v/v (ortho phosphoric acid buffer preparation; Take 1000ml of HPLC grade water and 1 ml orthophosphoric acid and pH adjested) as mobile phase, at a flow rate of 1.0 mL min-1. UV detection was performed at 221 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Simuvastatin and Ezetimibe were 2.190 and 3.515 min, respectively.
Calibration plots were linear over the concentration ranges 10–50 µg mL-1 and 10-50 µg mL-1 for Simvastatin and Ezetimibe, respectively. The Limit of detection was 2.0 and 6.31µg mL-1 and the quantification limit was 6.31 µg mL-1 and 2.99 µg mL-1 for Simvastatin and Ezetimibe, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.98% to 101.21%..

Keywords: Simvastatin,Ezetimibe, RP-HPLC,Validation.

INTRODUCTION

Simvastatin (SIM) butanoic acid, 2, 2-dimethyl-1, 2, 3,7, 8, 8a-hexahydro-3, 7-dimethyl-8- [2 (tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) -ethyl]-1-naphthalenyl ester, is a lipid-lowering agent that is derived synthetically from fermentation products of Aspergillus terreus1. After oral ingestion SIM, which is an inactive lactone, is hydrolyzed to the corresponding β-hydroxy acid that is an inhibitor of 3-hydroxy 3-methyl glutaryl – coenzyme A. (HMG- CoA) reductase. This enzyme catalyzes the conversion of HMG CoA to mevalonate, which is an early and rate limiting step in cholesterol biosynthesis2. Ezetimibe (EZ), 1- (4-Fluorophenly) – 3 (R)- [3-(4-fluorophenyl)- 3 (S) hydroxy propyl]-4 (S)–(4-hydroxy phenyl) – 2 azetidinones, is a therapeutically beneficial drug that works by inhibiting the protein transporters on small intestinal brush border, which brings about this active transport of cholesterol. In addition, it also inhibits phytosterol absorption3. EZ has no inhibitory effect on absorption of lipid soluble vitamins, triglycerides or bile acids, as do statins. This distinct mechanism of action results in a synergistic cholesterol lowering effect when used together with statins that inhibits cholesterol synthesis by liver4. Recently a combination of SIM and EZ has been launched in market. In this combination, EZ shows a synergistic effect with SIM. SIM was determined by several methods including gas chromatography–mass spectrometry (GC–MS)5, liquid chromatography with UV detection (LC–UV)6-8. EZ was determined with or without combination of several drugs by HPLC and spectrophotometrically9, 10. Literature survey revealed that no HPLC method has been reported yet for the analysis of these two drugs in combination without preliminary separation that makes it worthwhile to pursue the present work.

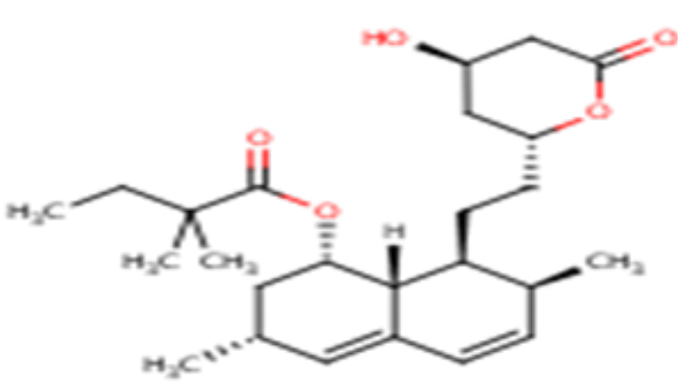


Figure-1: Molecular structure of Simvastatin

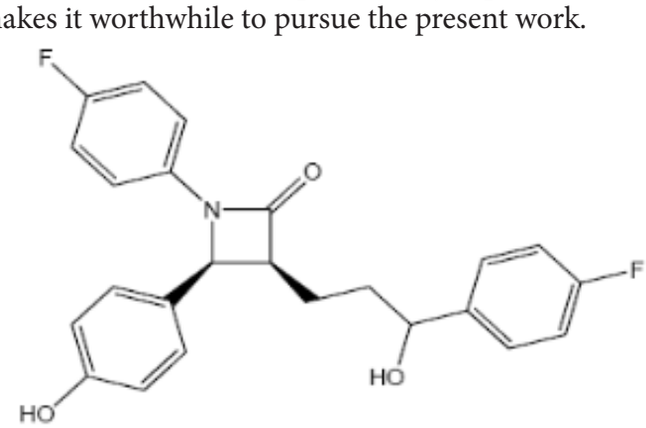


Figure-2: Molecular structure of Ezetimibe

MATERIALS AND METHODS:

Chemicals/ Reagents and Solvents

Simvastatin-10mg(Simcard 10) and Ezetimibe-10mg9(EzedocR)10 were obtained from, Rambaxy Laboratories Limited, .Himachal Pradesh and Hovero Labs Limited, Himachal Pradesh, respectively. Double Distilled Water (HPLC grade), Methanol(HPLC grade), Acetonitrile (HPLC grade), orthophosphoric acid and Potassium-dihydrogen phosphate were of reagent grade.

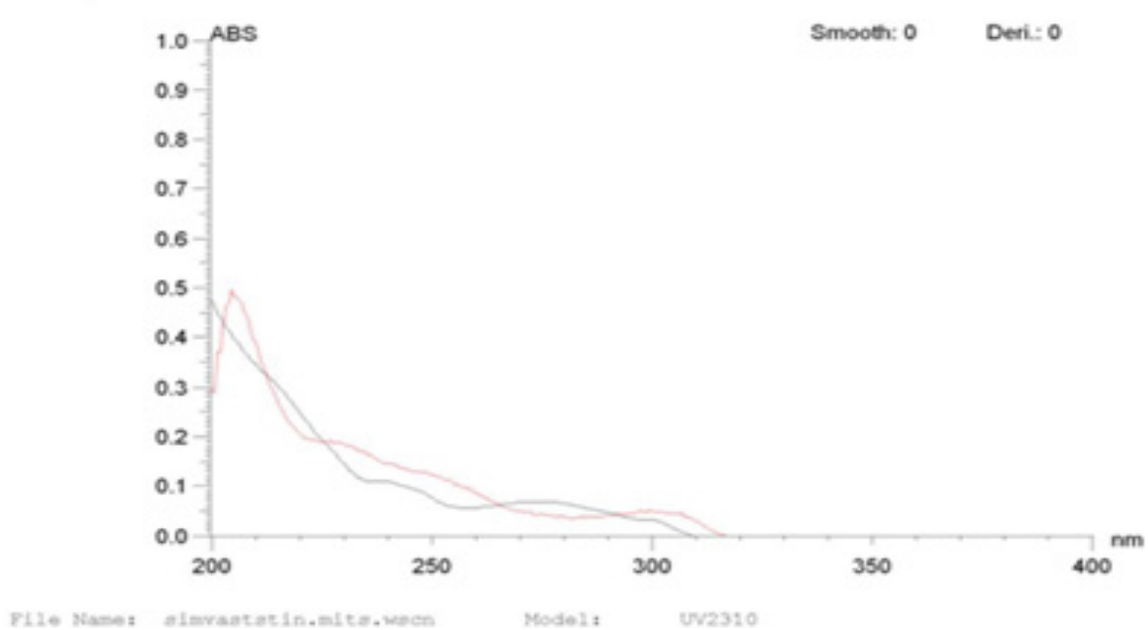
Instrumentation and Equipments

The HPLC analysis was accomplished on WATERS high pressure liquid chromatography outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20µL sample loop, X-terra C8 4.6mm x 150mm analytical column reversed-phase material of 5µ size and a 2487 model UV-Visible detector. All the parameters of HPLC were controlled by N 2000 chromatographic system software. Other instruments used were TECHCOMP UV-Vis spectrophotometer of model 2310, Shimadzu electronic balance of model XEX-200, ADWA of model AD102U digital pH meter and ENERTECH of model SE60US ultrasonic bath sonicator.3.3

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions Initially method development work was started by taking UV-visible spectra from 400-200 nm of simvastatin (10ppm) and Ezetimibe (10ppm) standard solutions. By observing the overlain spectra of standard solutions λmax 221 nm was taken for trials to develop HPLC method. The spectrum was show below

Figure-3. Isobestic point of Simvastatin and ezetimibe.



Chromatographic Conditions:

Mobile phase	:	Acetonitril : Ortho phosphoric acid(60:40v/v)
Column	:	Xterra (C8) (4.6mm x 150mm, 5µm)
Flow rate	:	1.0ml
Detector wavelength	:	221 nm
Retention time	:	Simvastatin-2.190 min Ezetimibe-3.515 min
Column temp	:	ambient.
Injection volume	:	20µl

Procedure for preparation of solution

Preparation of buffer

Take 1000ml of HPLC grade water. Dissolve 2.72 grams of Potassium di hydrogen phosphate salt and Adjusted the pH to 3.0 with orthophosphoric acid.

Preparation of mobile phase

A mixture of above prepared buffer 400 ml (40%), and 600 ml of HPLC grade Acetonitrile (60%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filterred through 0.45 µ filter under vacuum.

Diluent Preparation

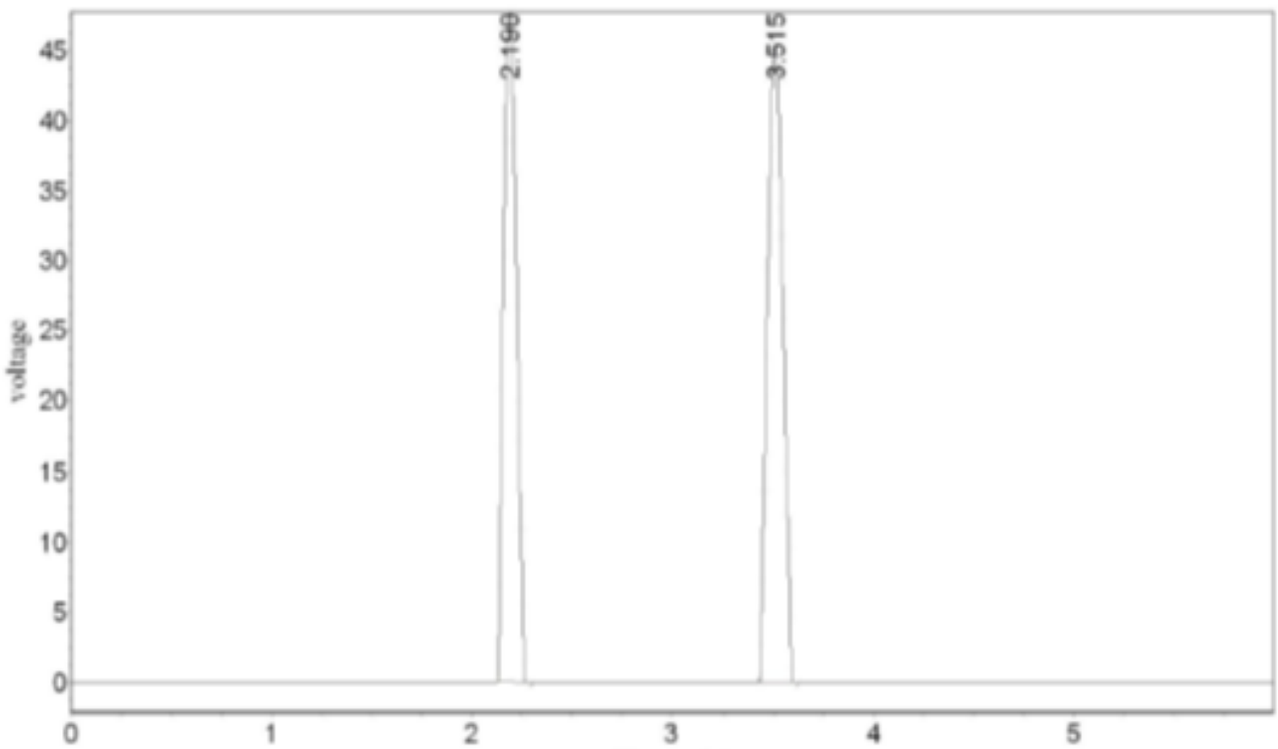
Use the Mobile phase as Diluent

ASSAY

Preparation of Standard Solution:

Accurately weighed and transferred 10mg of simvastatin and 10 mg of Ezetimibe working standard into a 100 ml clean dry volumetric flask and added about 70

Figure-4 Optimized Chromatogram For Simvastatin And Ezetimibe



ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)
From the above stock solution, 1 ml of the a solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation
Accurately weighed and transferred tablet powder equivalent to 10mg of simvastatin and 10 mg of Ezetimibe into a 100 ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)
From the above stock solution, 1ml of the solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent.

Procedure
20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Simvastatin and Ezetimibe peaks were measured. %Assay was calculated by using the formulae.

Calculation
Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where
AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = LABEL CLAIM mg/ml.

ANALYTICAL METHOD VALIDATION
The HPLC method was validated in accordance with ICH guidelines.

Accuracy:
Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of Simvastatin and Ezetimibe a known amount of standard drug powder of Simvastatin and Ezetimibe were added at 80%, 100% and 120 % level.

Precision:
The system precision of the method was verified by five replicate injections of standard solution containing Simvastatin and Ezetimibe . The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of Simvastatin and Ezetimibe.

Linearity:
The linearity was determined separately for Simvas-

tatin and Ezetimibe Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations.

Limit of detection and Limit of quantitation:

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness:

Robustness was evaluated by making deliberate variations in method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for Simvastatin and Ezetimibe

RESULTS:

Selection of Chromatographic Conditions and Optimization of Mobile Phase:

Mobile phase was optimized to separate Simvastatin and Ezetimibe using Symmetry C8 column (150 mm x 4.6 mm i.d., 5µm). Initially, ACN and phosphate buffer and methanol in the Equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (phosphate buffer, ACN in 40:60 % v/v) was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 1.0 ml/min-1. Under optimum chromatographic conditions, the retention time for Simvastatin and Ezetimibe was found to be 2.190 and 3.315 min, respectively when the detection was carried out at 221nm. A typical chromatogram of two drugs is shown in (Figure –4).

Table no.1 Accuracy data of Simvastatin and Ezetimibe

	Simvastatin			Ezetimibe		
	80%	100%	120%	80%	100%	120%
Inj-1	2315768	2506568	2841588	2315768	2506568	2841588
Inj-2	230415	2593805	2862840	2304125	2593805	2862840
Inj-3	233903	2589046	2859430	2332903	2589046	2859430
avg	2317599	2563139.7	2854619.3	317599	2563139.7	2854619.3
S.D	14476.08	4905.251	11413.543	14476.08	49050.51	11413.534
%RSD	0.62	1.91	0.40	0.62	1.91	0.40

Table No.2 Accuracy(Recovery) result for Simvastatin

Spike level	Area	Amount added(Std conc+working conc) (ppm)	Amount Found (ppm)	Amount recoverd (ppm)	%recoverd	Mean % recoverd
80%	2047651	54	54.36	24.36	101.5	99.98
100%	2196091	60	60.0	30.02	100.066	
120%	2393062	66	65.41	35.41	98.36	

Table No.3 Accuracy(Recovery) result for Ezetimibe

Spike level	Area	Amount added(Std conc+working conc) (ppm)	Amount Found(ppm)	Amount recoverd(ppm)	%recoverd	Mean % recoverd
80%	2367599	54	54.46	24.46	101.92	101.215
100%	2598473	60	60.02	30.02	100.066	
120%	2393062	66	65.41	35.41	101.66	

Table No:4 Results of Precision for Simvastatin

S.No	Injections	Area of Simvastatin
1	Injection-1	1030445
2	Injection-2	1031303
3	Injection-3	1021212
4	Injection-4	1017377
5	Injection-5	1031363
	Avarage	10226340
	Standard deviation	6154.39
	%RSD	0.59

Table No:5 Results of Precision for Ezetimibe

S.No	Injections	Area of Ezetimibe
1	Injection-1	1179915
2	Injection-2	1168003
3	Injection-3	1155515
4	Injection-4	1173587
5	Injection-5	1155954
	Avarage	1166594.8
	Standard deviation	10773.69
	%RSD	0.923

TABLE No:6 Results of Intermediate precision for Simvastatin and Ezetimibe

S.No	Injections	Area of Simvastatin	Area of Ezetimibe
1	Injection-1	1086110	1240329
2	Injection-2	1076922	1231744
3	Injection-3	1095482	1233601
4	Injection-4	1089521	1239973
5	Injection-5	1083763	1230438
	Avarage	1086359.6	1235217
	Standard Deviation	6875.41	4643.923
	%RSD	0.632	0.375

Table No:7 Area of different concentration of Simvastatin

S.No	Concentration(µg/ml)	Area of Simvastatin
1	10	226891
2	20	676546
3	30	1097595
4	40	1460083
5	50	1882919

TableNo:8 Area of different concentration of Ezetimibe

S.No	Concentration(µg/ml)	Area of Ezetimibe
1	10	26795
2	20	801471
3	30	130410
4	40	1798911
5	50	344965

Fig No:5 Linearity Graph of Simvasatin

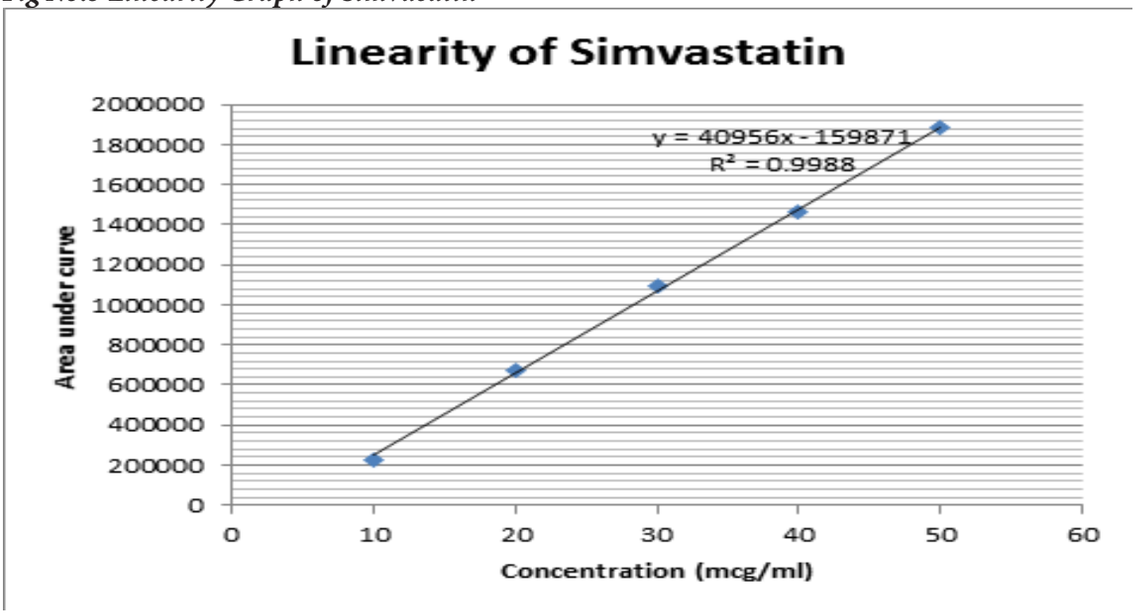


Fig No: 6 Linearity Graph of Ezetimibe

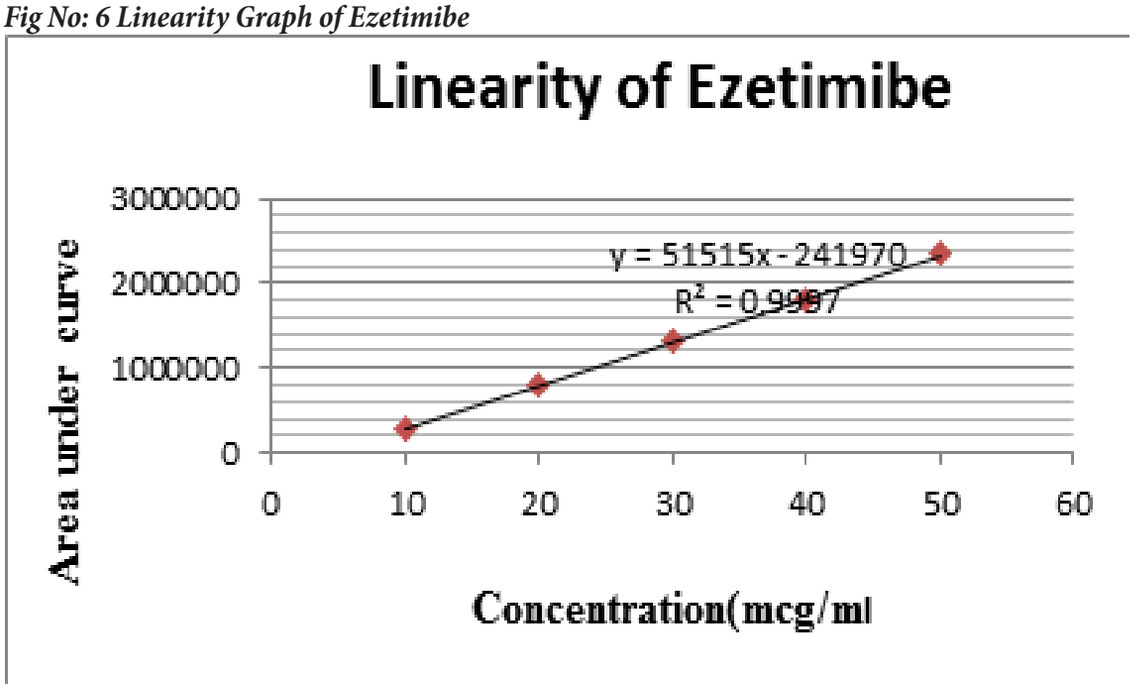


Table no :9 Results of LOD and LOQ

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Simvastatin	25865.34	40956	2.0	6.31
2	Ezetimibe	15434.88	51515	0.988	2.99

Table No :10 Robustness Result For Rosuvastatin And Ezetimibe At Different Condition

Sno		Simvastatin		Ezetimibe	
		RT	Area	RT	Area
1	Standard	2.12	10121207	3.512	1178228
2	Less low	2.37	1230806	3.81	1399394
3	More flow	2.23	96687	3.248	1093351
4	Less org	2.34	1232634	3.74	1398037
5	More org	2.29	100057	3.512	1178228

RESULTS AND DISCUSSION

Accuracy:

The accuracy of the method studied at three different concentration levels i.e. 80%, 100 % and 120 % showed acceptable % recoveries in the range of 99.98% for Simvastatin and 101.21% for Ezetimibe . The results are shown in Table 1,2&3

Precision:

The precision study was evaluated on the basis of % RSD value was found to be The RSD values for SIM and EZE were found to be 0.59% and 0.923% respectively Table -4&5

Linearity:

The linearity was determined separately for Simvastatin and Ezetimibe .Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in mobile phase and calibration curves were constructed by plotting peak area against the respective concentrations. The Simvastatin and Ezetimibe followed linearity in the concentration range of 10-50 µg ml-1 and 10-50 µg ml-1; respectively. The results are shown in Table 7&8.and Fig no 5 & 6

Limit of detection and Limit of quantitation:

The LOD for Simvastatin and Ezetimibe was found to be 2.0 and 0.98 µg/ml, respectively. The LOQ for Simvastatin and Ezetimibe was found to be 6.31and 2.99 µg/ml respectively. The low values of LOD and LOQ indicates high sensitivity of the method. The results are shown in Table 9.

Robustness study:

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The low value changes of theoretical plates, tailing factor indicating robustness of the method. The results are shown in Table 10.

Analysis of marketed tablet formulation:

3 replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of Simvastatin and Ezetimibe estimated were found to 100.19 % and 98.55%, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in Table 11.

System Suitability Test:

The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in Table 12.

CONCLUSIONS:

The proposed RP-HPLC method allows for accurate, precise and reliable measurement of SIM and EZ simultaneously in combined dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two-component tablet dosage form of SIM and EZ. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of SIM and EZ in multicomponent pharmaceutical preparation.

Table No:11 ASSAY RESULTS

Assay Results Drug	Amount present/tablet	% of Assay
Simvastatin	10mg	100.19
Ezetimibe	10mg	98.55

Table no :12 System Suitability Results For Simvastatin And Ezetimibe

S.No	Drug	Tailing factor	Theoritical plate for Column	Resolution
1	Simastatin	1.125	4.20	-
2	Ezetimibe	1.034	7857	7.794

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