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

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ABSTRACT

A new Simple, Precise, Fast and Accurate RP-HPLC Method has been developed and validated for simultaneous estimation of Ezetimibe and Simvastatin in bulk and combined dosage form. The method was carried out on the chromatographic separation was achieved by isocratic elution technique on (reverse phase) Primesil C18 column (250 mm x 4.6 mm ID, Particle size 5µm), using mobile phase composition of Methanol: Water (0.05%OPA) in the ratio of 90:10 v/v with a flow rate 1.0ml/min. The separation was observed at 239 nm. The retention time of Ezetimibe and Simvastatin were found to be 3.98 min and 7.91 min respectively. The Ezetimibe and Simvastatin followed linearity in the concentration range of 5-25µg/ml and 5-25µg/ml respectively with r^2=0.999 for both Ezetimibe and Simvastatin. The amount of both drugs estimated by the proposed method was found to be in good agreement with labelled claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combined dosage form.

KEYWORDS: Ezetimibe and Simvastatin, RP-HPLC, Validation.

INTRODUCTION

EZETIMIBE

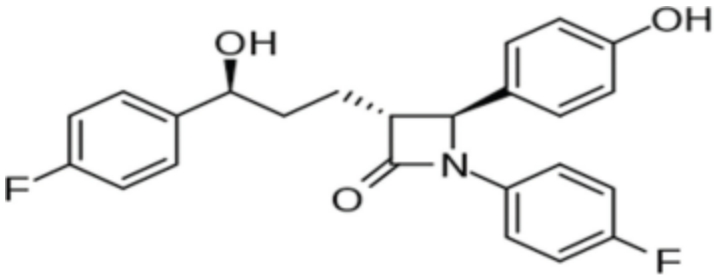

Fig No. 1: Chemical Structure Of Ezetimibe.

Ezetimibe[Fig no1] is an anti hyperlipidemic medication which is used to lower cholesterol levels. Chemically, Ezetimibe is (3R,4S) –1–(4 flurophenyl)–3-[(3S)-3- (4– flurophenyl)– 3 – hydroxypropyl] -4 –(4 hydroxyphenyl)azetidin – 2- one.[4] It localizes and appears to act at the blush border of the small intestine and inhibits the absorption of cholesterol, leading to decrease in the delivery of intestinal cholesterol to the liver.[4-5]

SIMVASTATIN

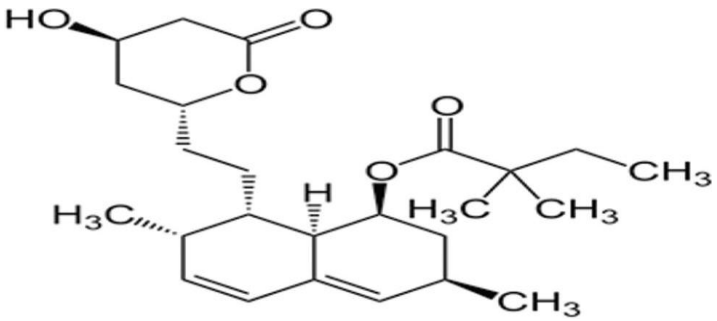

Fig No.2: Chemical Structure of Simvastatin.

Simvastatin[Fig no2] is a derivative of lovastatin and potent competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme. Simvastatin is [(1S,3R,7S,8S,8Ar) –8–[2–[(2R,4R)–4 – hydroxyl– 6–oxooxan -2 –yl] ethyl] – 3,7– dimethyl –1,2,3,7,8,8a– hexahydronaphthalene– 1– yl] 2,2–dimethyl butanoate.[4] In Simvastatin, the six membered lactone ring of simvastatin is hydrolysed *in vivo* to generate mevilonic acid and active metabolite structurally similar to HMG CoA (Hydroxymethylglutaryl CoA).Once hydrolysed, simvastatin competes with HMG – CoA for HMG – CoA reductase, a hepatic microsomal enzyme. Interference with the activity of this enzyme reduces the quality of mevalonic acid precursor of cholesterol.[4-6]

MATERIALS AND METHODS

Materials

Ezetimibe and Simvastatin was purchased from Swapnroop drug and pharmaceuticals. Commercial Tablet of Ezetimibe and Simvastatin named Simvotin EZ was purchased from local market. All other chemicals and reagents used are HPLC grade manufactured by Merck Ltd. India.

Instrument and chromatographic conditions

High performance liquid chromatography YounglineAcme9000 having autochro-3000 software containing primesilC_{18} column (250mm X 4.6mm, 5µm) was used for the study. UV-Spectrophotometer is used as a detector. The mobile phase used was Methanol: Water (0.05% Orthophosphoric acid) in the ratio of 90:10% v/v maintained at pH 3 with a flow rate 1.0 ml/min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed in Sonicator. The effluent was detected at 239 nm. The Column temperature was maintained at ambient and the volume of injection is 20 µl.

Preparation of mobile phase

The mobile phase was prepared by Mixing Methanol (90ml) and Water (10ml) and adjusted to the pH 3 with Ortho Phosphoric Acid. The prepared mobile phase was degassed by ultrasonication for 20 min so as to avoid the disturbances caused by dissolved gases. This degassed mobile phase was filtered through 0.45 µg membrane nylon filter to remove smaller particles that may be present in mobile phase.

Preparation of solutions

Standard stock solution

Standard stock solution prepared of accurate weight of 10mg of Ezetimibe and 10 mg of Simvastatin transfer into clean dry 10 ml volumetric flask add about the 9 ml Methanol Diluent and sonicate to dissolve in it completely and make volume up to the mark with the same solvent. (Stock were prepared) Then from that stock solution pipette out 0.1ml solution & dilute to 10 ml in volumetric flask. add about the mobile phase methanol: Water(90:10 v/v) in diluents the make volume mark to the 10 ml volumetric flask.

Working standard solution

1mL of standard stock solution was pipetted into 10mL volumetric flask and diluted up to the mark with diluent and filtered through 0.45µ Millipore Nylon filter to obtain concentration of 10 µg/ml.

EXPERIMENTAL

Study of spectra and selection of wavelength

UV Detector was selected and solution 10 µg/mL of Ezetimibe and Simvastatin was scanned in the range of 400 – 200nm. A fix concentration of analyte were analysed at different wavelengths. As per response of analyte, 239nm wavelength was selected. The UV Spectra of Ezetimibe and Simvastatin is as shown in Fig. 3.

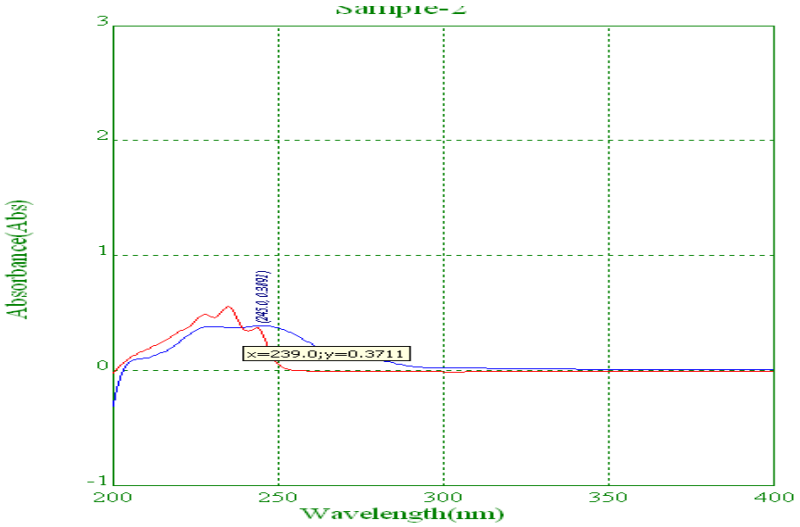

Fig No. 3: Overlain Spectra of Ezetimibe and Simvastatin.

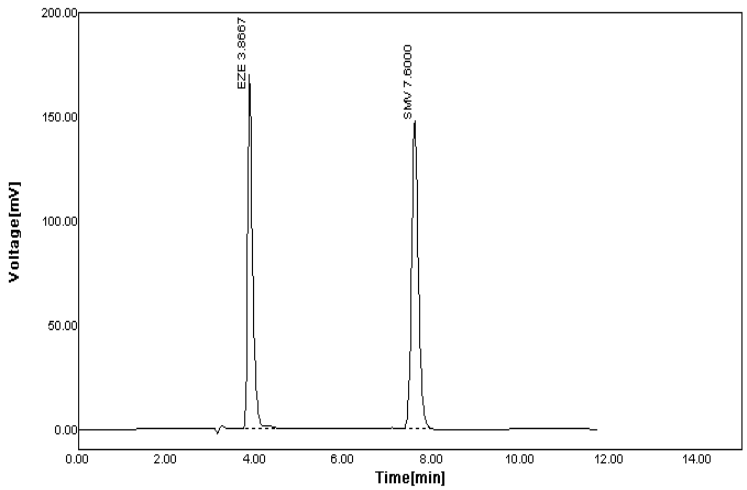

Fig No. 4: Chromatogram of standard Combination of Ezetimibe & Simvastatin

Analysis of marketed formulation

Procedure

For analysis of the tablet dosage form, 20 tablets were weighed individually and their average weight was determined after that they were crushed to fine powders and power equivalent to weight 173 mg of was weighed and transferred to 10 ml volumetric flask &. were dissolved in HPLC grade methanol. the solutions were shaken vigorously for 10 min and filtered through 0.45 µg nylon membrane filters. Then volume was made up to mark with methanol. From the above solutions 0.20 ml from Stock was taken and diluted to 10 ml with mobile phase to get a solution containing 20 µg/ml and 20 µg/ml respectively, so that the the solution contains Ezetimibe and Simvastatin in the proportions of 10:10 The amounts of Ezetimibe and Simvastatin per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Result is shown in (Table No. 1).

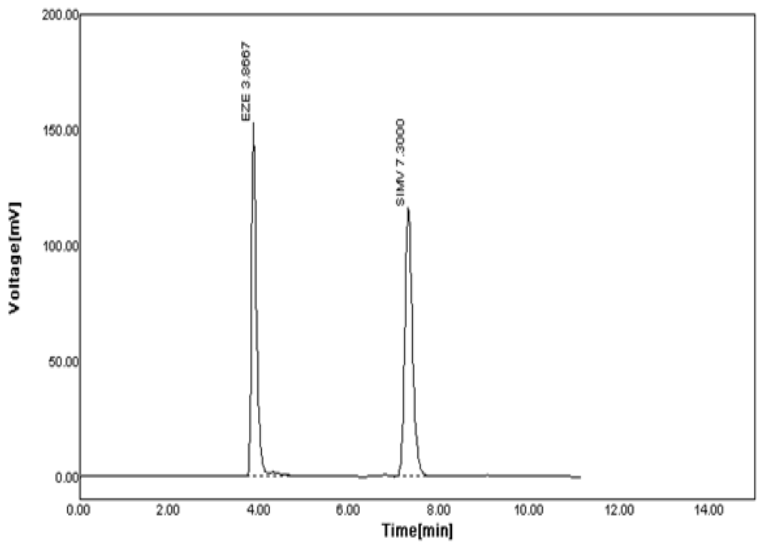

Fig. No. 5 Chromatogram of Ezetimibe and Simvastatin in tablet formulation.

TABLE 1. Analysis of marketed formulation.

Sr.no	Amount present in mcg		Amount found in mg		% Label claim	
	Ezetimibe	Simvastatin	Ezetimibe	Simvastatin	Ezetimibe	Simvastatinn
1	20	20	19.35	19.92	96.78	99.60
2	20	20	19.64	19.96	98.24	99.80
Mean	_	_	24.85	20.73	97.51	99.70
SD	_	_	0.21	0.03	0.35	0.64
%RSD	_	_	0.83	0.14	0.37	0.61

Analytical Validation Method

The method was validated in terms of the following parameters; linearity, accuracy, precision, repeatability, robustness, LOD, LOQ and system suitability parameters as per the ICH Guidelines.

1) Linearity

From Ezetimibe and Simvastatin standard stock solution, different working standard solution (5, 10, 15, 20, 25µg/ml) were prepared in mobile phase. Chromatogram was recorded. The plot of linearity plotted graphically as a function of analyte concentration is as shown in fig.6 and 7.

Table. 2. Linearity of Ezetimibe.

Sr. No.	Concentration µg/ml	Area Ezetimibe
1	5	250.42
2	10	558.99
3	15	904.09
4	20	1186.35
5	25	1538.38

Table. 3. Linearity of Simvastatin.

Sr. No.	Concentration µg/ml	Area Simvastatin
1	5	293.39
2	10	631.07
3	15	996.16
4	20	1377.55
5	25	1709.80

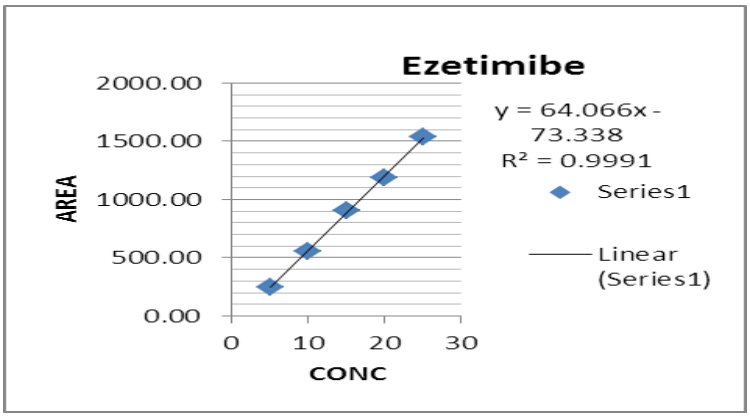

Fig.No.6: Calibration curve of Ezetimibe

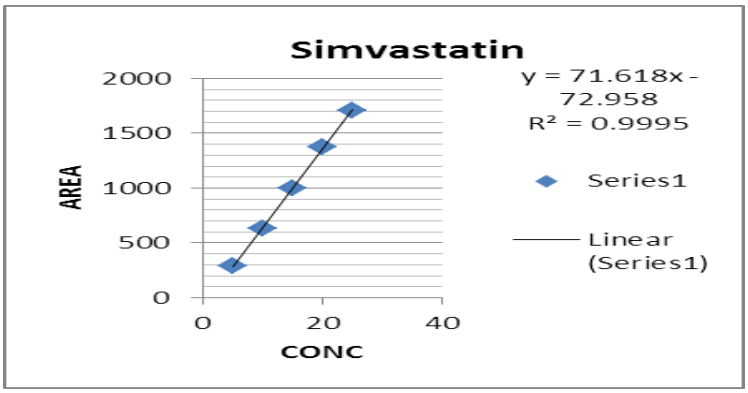

Fig.No.7: Calibration curve of Simvastatin

2) Accuracy

The accuracy study was performed at three different levels (80%, 100% and 120% of the test concentration). The mean % recoveries were found to be between 98–102% as required by ICH guidelines. The results of the recovery studies and its statistical validation data are given in Table No.4 and 5.

TABLE 4. Recovery studies of Ezetimibe and Simvastatin.

Level of Recovery (%)	80		100		120	
	EZE	SIM	EZE	SIM	EZE	SIM
Amount present (mg)	10 10	10 10	10 10	10 10	10 10	10 10
Amount of Std. Added (mg)	8 8	8 8	10 10	10 10	12 12	12 12
Amount Recovered (mcg)	8.09 7.87	7.67 9.75	9.52 9.67	10.15 10.30	12.18 12.01	11.75 11.91
% Recovery	101.15 99.31	98.19 98.65	95.21 96.72	101.56 103.09	101.55 100.96	97.96 99.26

***mean of each 3 reading.**

TABLE 5. Statistical Validation of Recovery Studies.

Level of Recovery (%)	Drug	Mean % Recovery*	Standard Deviation*	%RSD*
80	EZE	100.23	99.77	100.00
	SIM	98.42	0.33	0.33
100	EZE	95.97	1.07	1.11
	SIM	102.33	1.08	1.06
120	EZE	101.26	0.42	0.41
	SIM	98.61	0.92	0.93

***mean of each 3 reading.**

3) Precision

Precision of the analytical method is expressed as the series of the measurement. It was ascertained by replicate estimation of the drug by the proposed method as shown in (Table No.6).

Table No. 6: Result of Intra day and Inter day Precision for Ezetimibe & Simvasatatin.

Drug	Conc^{n} (µg/ml)	Intraday Precision			Interday Precision		
		Mean	SD	%Amt Found	Mean	SD	% Amt Found
Ezetimibe	10	556.99	9.33	98.39%	558.28	4.36	98.59%
	15	902.68	4.29	101.57%	905.82	4.65	101.89%
	20	1175.56	22.22	97.47%	1164.98	6.18	97.47%

Simvastatin	10	629.58	11.68	98.10%	635.58	6.02	98.94%
	15	976.96	6.60	97.74%	995.46	4.48	99.46%
	20	1369.80	0.94	100.73%	1366.44	2.79	100.50%

***Mean of each 3 reading**

4) System suitability parameters

System suitability tests are an intergral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time, number of theoretical plates, tailing factor and peak area were evaluated replicate five injections of the Ezetimibe & Simvastatin at a concentration of 5-25 µg/ml.) The result shown in below (Table No.7).

TABLE 7. Result of System Suitability Parameters.

System Suitability Parameters	*Mean of Five Determination System Suitability Parameters	
	EZE	**SIM**
Retention Time(min)	3.92 min	7.69 min
Area	975.5652	1072.14
Theoretical Plate Number	7869.18	1231.92
Tailing Factor	1.3721	1.2105
Resolution	0.0000	16.49

***Mean of each 5 reading.**

5) Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and wave length of drug peak was studied. The results of robustness studies are shown in (Table No.8).

TABLE 8. Result of Robustness Studies.

Parameter	Concenteration		Amount of detected (mean)		%RSD	
	EZE	**SIM**	**EZE**	**SIM**	**EZE**	**SIM**
Mobile Phase composition(91+9)	10	10	494.60	482.57	1.13	0.92
Mobile Phase composition(89+11)	10	10	553.96	536.21	0.94	1.61
Wavelength change(238nm)	10	10	452.90	548.69	0.78	0.93
Wavelength change(240nm)	10	10	535.69	511.49	1.19	1.71
FlowRate change (1.1ml)	10	10	493.11	461.89	0.72	0.44
FlowRate change (0.9ml)	10	10	605.72	605.56	1.08	1.05

6) Detection Limit (LOD)

The LOD is the lowest limit that can be detected. based on the S.D. deviation of the response and the slope, the detection limit was found to be Ezetimibe 0.21 µg/ml and Simvastatin 0.33 µg/ml.

TABLE 9. Result of Detection Limit.

Drug Name	LOD
Ezetimibe	0.21
Simvastatin	0.33

7) Quantification Limit (LOQ)

TABLE 10. Result of Quantification Limit.

Drug Name	LOQ
Ezetimibe	0.65
Simvastatin	1.02

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope, the quantification limit was found to be Ezetimibe 0.65 µg/ml and Simvastatin 1.02 µg/ml.

RESULTS AND DISCUSSION

In this method Linearity was studied as 5-25 µg/ml. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80,100 and 120% level. In this method precision was studied as repeatability and inter and intra-day variations for both drugs and both were found in limit. System suitability parameters were satisfactory and the theoretical plates were obtained above 2000 The % recovery, robustness, LOD, LOQ, parameter data were presented in (**Table 1-10).**

CONCLUSION

The developed was found to be simple, precise, economic, fast. Further, the developed method is simple and can usually be used for estimation of both these drugs in their combined dosage form. This method is used for routine analysis of drugs in bulk and Combined dosage form.

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