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Development and Validation of RP- HPLC Analytical Assay Method for Ezetemibe and Simvastatin Tablet

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ABSTRACT

A simple, rapid, RP-HPLC method was developed and validated for simultaneous estimation of Ezetemibe and Simvastatin in the combined pharmaceutical dosage form. The separation of Ezetemibe and Simvastatin was successfully achieved. Column was used chromosilC_{18}having 250 x 4.6mm internal diameter and mobile phase was methanol: acetonitrile 0.1%orthophosphoric Aid in the ratio of (75:25:05 v/v/v) used at a flow rate of 0.5-1.5ml/mint for optimum separation. The elute was monitored at 243 nm. The retention time of Simvastatin and Ezetemibe was found to be 6.10 and 3.35 respectively. Results show that the proposed method is highly accurate and the method was validated according to ICH guidelines. Determination of the different analytical parameter such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) was done.

INTRODUCTION

Ezetemibe is a class of medication called cholesterol-lowering medications used to reduce the amount of cholesterol (a fat-like substance) and other fatty substances in the blood.[1] It may be used alone or in combination with an HMG-CoA reductase inhibitor (statin).[2]Ezetemibe It acts by decreasing cholesterol absorption in the intestine Ezetemibe[3] is the only add-on to statin therapy that has successfully shown cardiovascular benefit when combined with stain.[3]

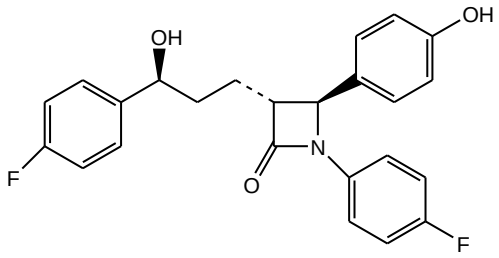


Figure 1.Chemical Structure of Ezetemibe[3]

Systematic (IUPAC) name: -

(3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl) hydroxypropyl]-4-(4-hydroxyphenyl) azetidine-2-one

Molecular Formula: - $C_{24}H_{21}F_2NO_3$

Mol. mass: - 409.4

Routes: - Oral

Side effects include gastrointestinal disturbances, headache, fatigue, myalgia; rarely arthralgia, hypersensitivity reactions (including rash, angioedema, and anaphylaxis), hepatitis; and very rarely pancreatitis, cholelithiasis, cholecystitis, thrombocytopenia, raised creatine kinase, myopathy, and rhabdomyolysis.

SIMVASTATIN:

Simvastatin is a hypolipidemic drug used with exercise, diet, and weight-loss to control elevated cholesterol or hypercholesterolemia Simvastatin belongs to a group of drugs called HMG CoA reductase inhibitors, or "statins." It reduces levels of "bad" cholesterol (low-density lipoprotein, or LDL) and triglycerides in the blood while increasing levels of "good"

cholesterol (high-density lipoprotein, or HDL) [4]. Simvastatin is a synthetic derivative of a fermentation product of Aspergillus terreus.

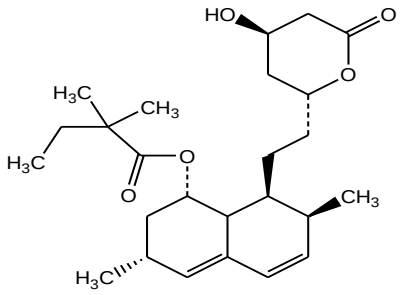


Figure 2: Chemical Structure of Simvastatin[4]

Systematic (IUPAC) name:- 1S, 3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2- yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate

Molecular Formula: - $C_{25}H_{38}O_5$

Mol. Mass: - 418.6

Routes: - Oral

Simvastatin, which is an inactive lactone, is hydrolyzed to the corresponding β-hydroxy acid form. This is an inhibitor of 3-hydroxy-3- methyl glutaryl-coenzyme A (HMG-CoA) reductase Simvastatin is also used to lower the risk of stroke, heart attack, and other heart complications in people with diabetes, coronary heart disease, or other risk factors. The primary uses of simvastatin are for the treatment of dyslipidemia and the prevention of cardiovascular disease Common side effects may include abdominal pain, diarrhea, indigestion, and a general feeling of weakness. Rare side effects include joint pain, memory loss, and muscle cramps.

MATERIALS & METHODS[5-6]:-

Chemicals and Reagents

Ezetemibe and Simvastatin as pure standard reference drugs were purchased from Ranbaxy Laboratory, (M.P.) Acetonitrile, Methanol and Orthophosphoric acid (all HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai, India.

Instrumentation

An isocratic PEAK HPLC instrument with a Hypersil C_{18} column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with an LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data were analyzed by using PEAK software. UV-2306 Spectrophotometer as used for wavelength checking.

Experimental Condition

The flow rate of the mobile phase was changed from 0.5 – 1.5 ml/min for optimum separation. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min at 25 ± 2ºC. For analysis the most suitable mobile phase was found to be Methanol, Acetonitrile and 0.1% Orthophosphoric Acid 75:25:05 Detection was carried out at a wavelength of 243 nm.

Preparation of Mobile Phase

Methanol, Acetonitrile and 0.1% Orthophosphoric acid of HPLC grade were mixed, filtered and degassed in such a way that the final volume consisted of these in the ratio 75:25:05 respectively, whose pH was found to be to 5.6

Preparation of mixed standard solution

Ezetemibe and Simvastatin (1mg/ml) standard stock solutions were prepared using the mobile phase as a solvent. Aliquots of mixed standard solutions of Ezetemibe and Simvastatin were diluted in the mobile phase to get a final concentration of 50-100 ppm.

Preparation of sample solution of pharmaceutical formulation

Pharmaceutical form containing 10 mg of Ezetemibe and 10 mg of Simvastatin was weighed and dissolved in 25 ml of mobile phase and sonicated for 15 min. Using methanol the volume was made up to 50 ml and filtered through a 0.45µ membrane filter

Recording of chromatograms

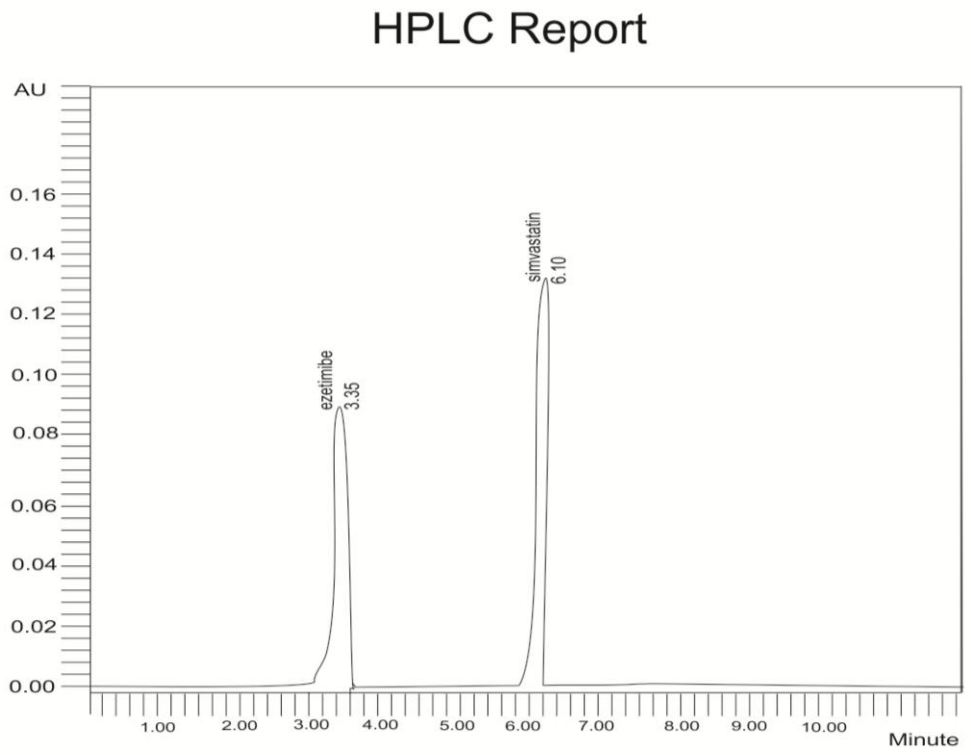
After stabilization of the baseline with the optimized chromatographic conditions standard solutions containing 50-100 ppm of Ezetemibe and Simvastatin were injected. The retention

time of Ezetemibe and Simvastatin were found to be 3.35 and 6.10 mints respectively. Likewise for sample solution chromatograms were recorded. Calibration curves were plotted using peak area retentions of standard drug peaks against the concentration of corresponding standard solutions.

RESULT AND DISCUSSION:-

Estimation[2]:-

An RP-HPLC method was developed for the simultaneous estimation of Ezetemibe and simvastatin in combined dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The standard and sample solution was prepared and chromatograms were recorded.



ID	Name	Retain.T	Height	Area	Conc.	Tail.Factor	Theo.Plate
1	Ezetimibe	3.35	51016	271383	43.542	1.91	7685
2	Simvastatin	6.10	65225	366340	56.458	1.54	24006
	Sum:		116241	637723	100.00		

Graph 1-Typical chromatogram of standard Ezetemibe and Simvastatin[2]

Method validation

The method was validated by determining linearity, precision, accuracy, specificity, ruggedness, and robustness by analyzing 50-100 ppm of both Ezetemibe and Simvastatin.

Table-1: Optimized Chromatographic Conditions for Estimation of Ezetemibe and Simvastatin[5]

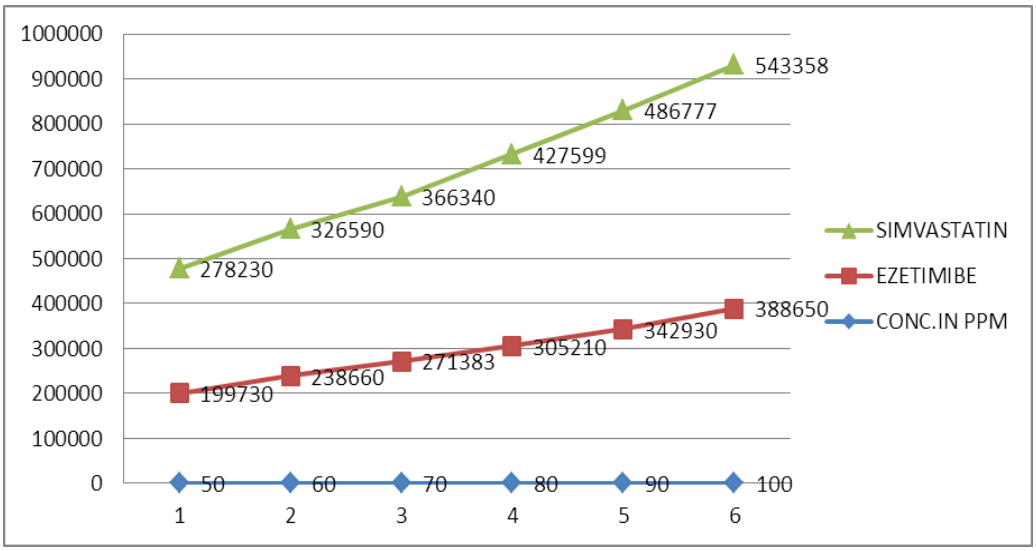
S.NO.	TEST	RESULT
	H.P.L.C. Condition	
1	ELUTION	ISOCRATIC
2	A.P.I CONC.	70ppm
3	MOBILE PHASE	Methanol: Acetonitrile: 0.1% Orthophosphoric Acid (75:25:05)
4	pH	5.6
5	COLUMN	C_{18}
6	WAVELENGTH	243nm
7	FLOW	1ml/min
8	RUNTIME	10min
9	RETENTION TIME	Ezetemibe 3.35 Simvastatin 6.10
10	AREA	Ezetemibe 271383 Simvastatin 366340
11	TH.PLATES	Ezetemibe 7685 Simvastatin 24006
12	TAILING FACTOR	Ezetemibe 1.91 Simvastatin 1.54
13	PUMP PRESSURE	9.8psi

Linearity:-

The linearity of the response for Ezetemibe and Simvastatin assay method was determined by preparing and injecting standard solutions of Ezetemibe and Simvastatin. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied with correlation coefficient (r^2) value, slope and intercept as

Table-2: Linearity result[12]

S. NO.	CONC. IN PPM	EZETEMIBE	SIMVASTATIN
1	50	199730	278230
2	60	238660	326590
3	70	271383	366340
4	80	305210	427599
5	90	342930	486777
6	100	388650	543358



Graph 2: Calibration plot for Ezetemibe and Simvastatin[6]

Table-3: Regression analysis of the calibration curve[12]

Parameters	Ezetemibe	Simvastatin
Calibration range (ppm)	50-100	50-100 ppm
Intercept	3280	1060
Slop	3830	5380.76
Correlation coefficient	0.999	0.999

Precision:-

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Ezetemibe and Simvastatin combined test solution in the same equipment at a concentration value of 70 ppm on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak areas of the drugs were determined and precision as % RSD was reported.

Table-4: Intraday precision[12]

S. No.	Concentration	Ezetemibe peak area	Simvastatin peak area
1	70 PPM	271263	366373
2	70 PPM	272239	365320
3	70 PPM	272490	367820
4	70 PPM	271695	365194
5	70 PPM	272065	365325
6	70 PPM	273382	367630
		% R.S.D. = 0.23	%R.S.D. = 0.34

Table-5: - Inter-day precision[12]

S. No.	Concentration	Ezetemibe peak area	Simvastatin peak area
1	70 PPM	274676	365621
2	70 PPM	266650	365185
3	70 PPM	268690	368724
4	70 PPM	271405	365325
5	70 PPM	272491	365189
6	70 PPM	268539	366897
		% R.S.D. = 1.54	%R.S.D. = 0.52

Table 6: System suitability of Ezetemibe and Simvastatin[12]

PARAMETERS	EZETEMIBE	SIMVASTATIN
Theoretical plates(N)	7685	24006
Retention time(min)	3.35	6.10
Tailing factor	1.91	1.54
LOD (ppm)	1.2ppm	0.25ppm
LOQ (ppm)	4ppm	0.8ppm
R.S.D. (%)	0.75	0.4

Recovery:-

The recovery of the standard solutions was done by adding them to pre-analyzed sample solution at different levels i.e. 50%, 100%, and 150% separately to study the accuracy of the above method. The corresponding results were recorded

Table 7: Recovery of Ezetemibe and Simvastatin Specificity [12]

Recovery	Conc. Of Sample	Ezetemibe	Simvastatin	Ezetemibe % of recovery	Simvastatin %of recovery
50%	50ppm	50.31	50.15	100.56	100.21
75%	75ppm	74.52	74.75	99.90	99.9
100%	100ppm	99.92	100.34	99.92	100.34

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Ezetemibe and Simvastatin. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Ezetemibe and Simvastatin.

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

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines.

Robustness:-

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above

Table 8: Robustness study

Parameter	Modification	Ezetemibe area	Simvastatin area	Ezetemibe % of recovery	Simvastatin % of recovery
Standard		271383	366340		
MP	MeOH;CAN;0.1%OPA(75;25;5)	273340	366690	0.73	0.09
pH	5.2	270654	366320	0.225	0.07
Wavelength	248nm	270693	365467	00.25	0.247

Analysis of marketed formulations

The validated HPLC method was adopted for the quantification of Ezetemibe and Simvastatin in their combined pharmaceutical dosage form and the typical chromatograms of the formulation are shown in fig. The results of the analysis are given in Table 8. The contents of the pharmaceutical dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of Ezetemibe and Simvastatin in pharmaceutical dosage forms.

Table-9: Formulation[5]

Drug	Formulation	Dosage	Sample Conc.	Drug estimated	% of drug estimated
Ezetemibe	Vytorin	10mg	70ppm	68.57	99.80
Simvastatin	Vytorin	10mg	70ppm	68.65	99.61

CONCLUSION

The proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of Ezetemibe and Simvastatin in combined pharmaceutical dosage formulations. The method was validated and found to be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Ezetemibe and Simvastatin in the pharmaceutical dosage form. The percentage of recovery shows that the method is free from the interference of the excipients used in the formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to the cost-effective chromatographic method.

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