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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF EZETIMIBE AND SIMVASTATIN IN COMBINED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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Abstract

A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Ezetimibe and Simvastatin in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Chromosil C-18,column having 250 x 4.6mm internal diameter with a mixture of Methanol:Acetonitrile:0.1%Orthophosphoric Aid in the ratio of 75:20:05 (v/v/v) as mobile phase. Determination of the different analytical parameters such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The average recovery was found to be 99.88 and 100.12 for Ezetimibe and Simvastatin respectively. The proposed method is highly sensitive, precise and accurate, which was evident from the LOD value of 1.2ppm and 0.25ppm for Ezetimibe and Simvastatin respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulations of Ezetimibe and Simvastatin.

Key Words: Ezetimibe, Simvastatin, HPLC, Development, Validation.

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Introduction

Ezetimibe is a drug that lowers cholesterol. It acts by decreasing cholesterol absorption in the intestine. It may be used alone (marketed as Zetia or Ezetrol), when other cholesterol-lowering medications are not tolerated, or together with statins (e.g.,ezetimibe/simvastatin, marketed as Vytorin and Inegy) when statins alone do not control cholesterol.

Even though ezetimibe decreases cholesterol levels, the results of two major, high-quality clinical trials (in 2008 and 2009) showed that it did not improve clinically significant outcomes, such as major coronary events, and actually made some outcomes, such as artery wall thickness, worse. Indeed, a panel of experts concluded in 2008 that it should "only be used as a last resort".[1] In one of those studies, a head-to-head trial in 2009, a much less expensive medication (extended-release niacin) was found to be superior. Ezetimibe actually increased the thickness of artery walls (a measurement of atherosclerosis) and caused more major cardiovascular events.[2] A more positive view of the benefits of Ezetimibe is offered by Britain's NICE statement which however was published in 2007 and may not have been updated to reflect the results of the above mentioned trials.[3]

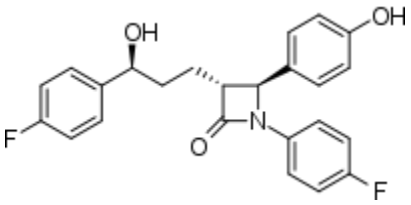


Figure 1: Stricture of Ezetimibe

Ezetimibe localises at the brush border of the small intestine, where it inhibits the absorption of cholesterol from the intestine. Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tract epithelial cells[4] as well as in hepatocytes.[5] In addition to this direct effect, decreased cholesterol absorption leads to an upregulation of LDL-receptors on the surface of cells and an increased LDL-cholesterol uptake into cells, thus decreasing levels of LDL in the blood plasmawhich contribute to atherosclerosis and cardiovascular events.[6]

Common adverse drug reactions (≥1% of patients) associated with ezetimibe therapy are headache and/or diarrhea (steathorrea). Infrequent adverse effects (0.1–1% of patients) include: myalgia and/or raised liver function test (ALT/AST) results. Rarely (<0.1% of patients), hypersensitivity reactions (rash, angioedema) or myopathy may occur.[7] Side-effects include gastro-intestinal disturbances; headache, fatigue; myalgia; rarely arthralgia, hypersensitivity reactions (including rash, angioedema, and anaphylaxis, hepatitis; very rarely pancreatitis, cholelithiasis, cholecystitis, thrombocytopenia, raised creatine kinase, myopathy, and rhabdomyolysis.[8]

Simvastatin is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia. It is a member of the statin class of pharmaceuticals.

Simvastatin is a synthetic derivate of a fermentation product of Aspergillus terreus. The drug is marketed under the trade name **Zocor**, as well as generically.

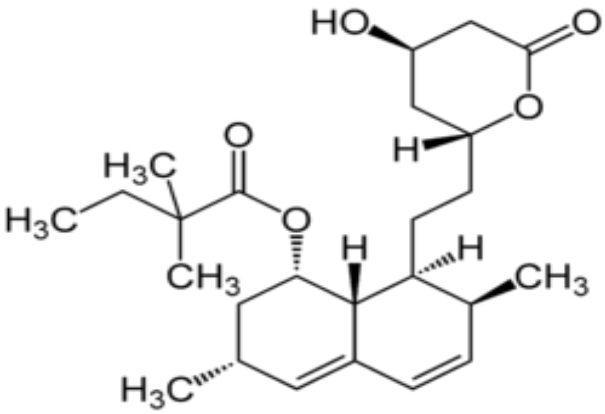


Figure 2: Stricture of **Simvastatin**

The primary uses of simvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease.[1] It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels.[9]

Common side effects (>1% incidence) may include abdominal pain, diarrhea, indigestion, and a general feeling of weakness. Rare side effects include joint pain, memory loss, and muscle cramps.[10] Cholestatic hepatitis, hepatic cirrhosis, rhabdomyolysis and myositis have been reported in patients receiving the drug chronically.[11]

Method and Material

Chemicals and Reagents

Ezetimibe and Simvastatin as pure standard reference drugs were purchased from Reddy's Laboratory, Hyderabad and pharmaceutical formulation from local market were used for this present study. Acetonitrile, Methanol and Orthophosphoric acid (all HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai, India.

Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Simvastatin and Ezetimibe , an isocratic PEAK HPLC instrument with Hypersil C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a 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 was analyzed by using PEAK software. UV-2306 Spectrophotometer was used for wavelength checking. Denver analytical Balance was used to weigh the drug.

Experimental Condition

Flow rate of the mobile phase was changed from 0.5 – 1.5 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min at 25 ± 2^{oc}. For analysis the most suitable mobile phase

was found to be Methanol, Acetonitrile and 0.1% Orthophosphoric Acid 75:20:05 Detection was carried out at wavelength of 243 nm.

Preparation of Mobile Phase

For the preparation of mobile phase suitable for the present determination 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 75:20:05 respectively, whose pH was found to be to 5.6

Preparation of mixed standard solution

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

Preparation of sample solution of pharmaceutical formulation

Pharmaceutical form containing 10 mg of Ezetimibe 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 0.45µ membrane filter. The final mixed sample solution corresponding to 70 ppm of Ezetimibe and 70 ppm of Simvastatin was prepared.

Recording of chromatograms

After stabilization of the base line with the optimized chromatographic conditions standard solutions containing 50-100 ppm of Ezetimibe and Simvastatin were injected and the corresponding chromatograms were recorded. Retention time of Ezetimibe and Simvastatin were found to be 3.30 and 6.17 mins respectively. Likewise for sample solution chromatograms were recorded. Calibration curves were plotted using peak area retentions of standard drug peaks against concentration of corresponding standard solutions.

Results and discussion

Method validation

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

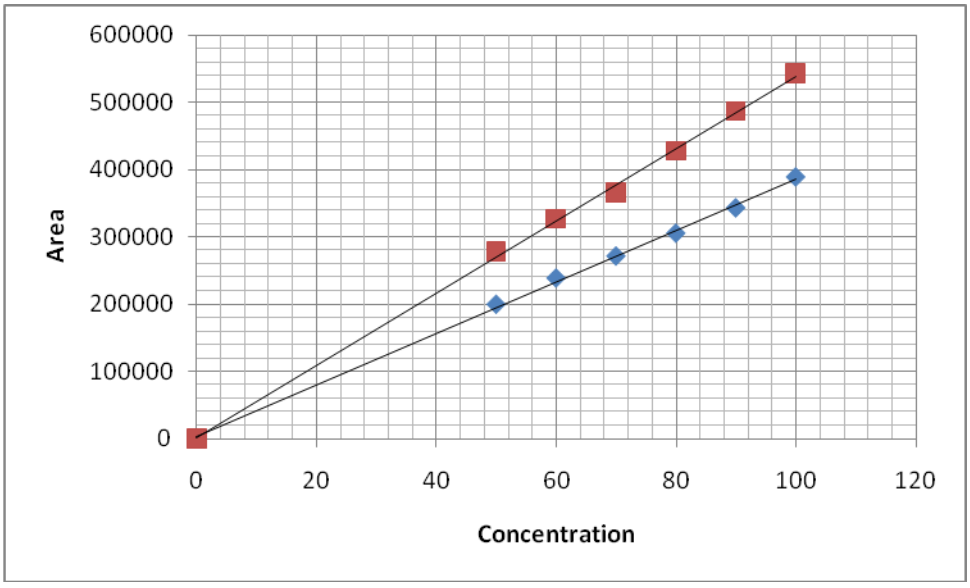
S.NO	TEST	RESULT
	H.P.L.C CONDITIONS	
1	ELUTION	ISOCRATIC
2	A.P.I CONC	70ppm
3	MOBILE PHASE	Methanol:Acetonitrile:0.1%Orthophosphoric Aid

S.NO	TEST	RESULT
		75:20:5
4	PH	5.6
5	COLUMN	C_{18}
6	WAVE LENGTH	243nm
7	FLOW	1ml\min
8	RUNTIME	10min
9	RETENSION TIME	Ezetimibe 3.30 Simvastatin 6.17
10	AREA	Ezetimibe 271253 Simvastatin 366363
11	TH.PLATES	Ezetimibe 7684 Simvastatin 24004
12	TAILING FACTOR	Ezetimibe 1.90 Simvastatin 1.53
13	PUMP PRESURE	9.8psi

Table 1: Optimized chromatographic conditions for estimation of Ezetimibe and Simvastatin

Linearity

The linearity of the response for Ezetimibe and Simvastatin assay method was determined by preparing and injecting standard solutions of Ezetimibe 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 shown in table 3.



Graph 1: Calibration Plot for Ezetimibe and Simvastatin

S.NO	CONC IN PPM	EZETIMIBE	SIMVASTATIN
1	50	199726	278226
2	60	238655	326584
3	70	271253	366363
4	80	305211	427588
5	90	342830	486767
6	100	388644	543348

Table.2: Linearity results

Parameters	Ezetimibe	Simvastatin
Calibration range (ppm)	50-100	50-100 ppm
Intercept	3285	1063
Slope	3829	5380.96
Correlation coefficient (r^2)	0.999	0.999

Table 3: Regression analysis of the calibration curve

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 Ezetimibe 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.

S.NO	CONCENTRATION	Ezetimibe peak area	Simvastatin peak area
1	70 PPM	271253	366363
2	70 PPM	272219	365322
3	70 PPM	272481	367819
4	70 PPM	271685	365174
5	70 PPM	272051	365315
6	70 PPM	273272	367534
		%R.S.D = 0.25	%R.S.D = 0.32

Table.4 Intraday precision

S.NO	CONCENTRATION	Ezetimibe peak area	Simvastatin peak area
1	70 PPM	274666	365521
2	70 PPM	266547	365174
3	70 PPM	268688	368714
4	70 PPM	271303	365315
5	70 PPM	272481	365174
6	70 PPM	268039	366697
		%RSD = 1.23	%RSD = 0.4

Table.5 Inter day precision

Parameters	Ezetimibe	Simvastatin
Theoretical plates (N)	7684	24004
Retention time (min)	3.30	6.17
Tailing factor	1.90	1.53
LOD (ppm)	1.2ppm	0.25ppm
LOQ (ppm)	4ppm	0.8ppm
R.S.D. (%)	0.750.35	0.4

Table 6.System suitability and validation parameters

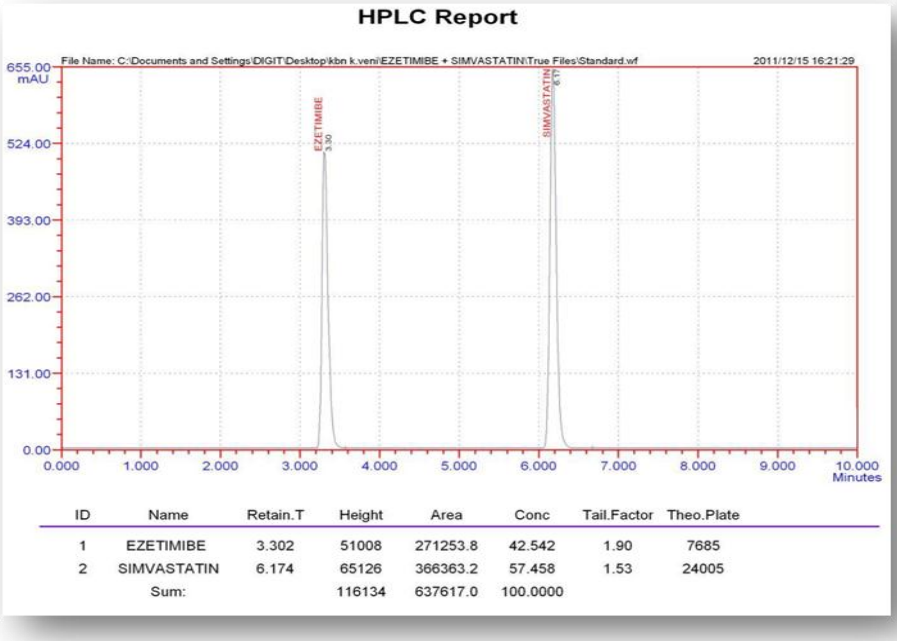


Figure.2 Typical chromatogram of standard Ezetimibe and Simvastatin

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.

Recovery	Conc. of sample	EZETIMIBE	SIMVASTATIN	EZETIMIBE % of recovery	SIMVASTATIN % of recovery
50%	50ppm	50.33	50.10	100.66	100.20
75%	75ppm	74.92	74.85	99.89	99.8
100 %	100ppm	99.1	100.35	99.1	100.35

Table.7 Recovery of Olmesaartan, Simvastatin

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Ezetimibe 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 Ezetimibe 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.

PARAMETER	**MODIFICATION**	**EZETIMIBE AREA**	**SIMVASTATIN AREA**	**EZETIMIBE %OF CHANGE**	**SIMVASTATIN % OF CHANGE**
Standard	…….	271253	366363	……..	……..
MP	MeOH:CAN:0.1%OPA 70:25:5	273238	366694	0.73	0.09
PH	5.1	270644	366121	0.225	0.07
Wavelength	249nm	270583	365457	00.25	0.247

Table.8 Robustness study

Analysis of marketed formulations

The validated HPLC method was adopted for the quantification of Ezetimibe and Simvastatin in their combined pharmaceutical dosage form and the typical chromatograms of the formulation are shown in fig. The results of 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 Ezetimibe and Simvastatin in pharmaceutical dosage forms.

Drug	FORMULATION	DOSAGE	SAMPLE CONC	DRUG ESTIMATED	% OF DRUG ESTIMATED
EZETIMIBE	VYTORIN	10 mg	70 ppm	69.87	99.81
SIMVASTATIN	VYTORIN	10 mg	70 ppm	69.71	99.586

Table.9 Formulation

Conclusion

The proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of Ezetimibe 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 Ezetimibe and Simvastatin in the pharmaceutical dosage form. The percentage of recovery shows that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

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