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Original Article

Quantification and Validation of Simvastatin and Ezetimibe in Bulk Drugs and Combined Dosage Form by Reverse Phase Liquid Chromatographic Method (RPLC)

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ABSTRACT

A simple, time saving, precise and cost effective reverse phase high performance liquid chromatographic (RP-HPLC) method developement was achieved for the determination and estimation of simvastatin and ezetimibe in its pure form and combined formulation. Separation was achieved by using Zorbax (100 × 4.6 mm, 5µ) C_{18} column with mobile phase consisted of acetonitrile and methanol in a ratio of 60:40 (v/v). The separation was observed at 232 nm with flow rate adjusted to 1 ml/min .simvastatin and ezetimibe were retained at 9.603 and 3.861 minutes successively. Validation was done for the developed method based upon different parameters like linearity, accuracy, precision, limit of detection and limit of quantitation. Simvastatin and ezetimibe obey Beer-Lambert's law in the range of 20.0-160 µg/ml and 5-40 µg/ml respectively. The % recoveries of simvastatin and ezetimibe were found to be 101.25% and 102.03% respectively from the tablet formulation. The limit of detection of simvastatin and ezetimibe were found to be 1.34 µg/ml and 0.253 µg/ml successively. The limit of quantitation of simvastatin and ezetimibe were found to be 4.489 µg/ml and 0.846 µg/ml successively. The established method is suitable for simultaneous estimation of simvastatin and ezetimibe in their pure forms and combined formulation.

Key words: Simvastatin, Ezetimibe, RP-HPLC, Simultaneous estimation.

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INTRODUCTION

Simvastatin chemically known as 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 (Figure 1), is an anti-lipidemic drug which is derived synthesized from fermentation products of *Aspergillus terreus.*[1] Simvastatin mainly used for the treatment and management of dyslipidemia and the prevention of cardiovascular disease.[2] It is instructed to use only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels.[3] General adverse reactions may include abdominal pain, diarrhoea, indigestion, and a general feeling of weakness. Rare side effects include joint pain, memory loss, and muscle cramps.[4] Cholestatic hepatitis, hepatic cirrhosis, rhabdomyolysis and myositis have been reported in patients receiving the drug chronically.[5] Ezetimibe (Figure 2) is a drug that decreases cholesterol. It decreases absorption of cholesterol in the intestine. It may be used alone (marketed as Zetia or Ezetrol), when other cholesterol lowering medications are not tolerated, or simultaneously with statins (ex-simvastatin/ezetimibe marketed as vytorin) when statins alone don't suppress cholesterol.[2] Although ezetimibe controls cholesterol, the outcomes of two clinical trials (2008 and 2009) proved that it was not having any improvement, like major coronary events, and shown some outcomes, like thickening of artery wall, worse. Eventually, a panel of experts concluded in 2008 that it should "can be the last resort".[6] Simvastatin was estimated by several methods including liquid chromatography with UV detection (LC–UV)[7-9], gas chromatography-mass spectrometry (GC-MS).[10] Ezetimibe was estimated alone or without combination of several drugs by high performance liquid chromatography and spectrophotometrically.[11,12]

Literature investigations reveal some HPLC methods have been reported for the estimation of these two drugs in combined dosage forms. Preliminary separation enforces pursuing of present research work.

MATERIALS AND METHODS

Materials, reagents and instrumentation

Simvastatin and Ezetimibe were gifted by Aurobindo Pharmaceuticals, Hyderabad, Andhra pradesh. HPLC grade Methanol and acetonitrile were purchased from Desai chemicals, Visakhapatnam. Waters HPLC 4000-separation module. Millennium software with PDA detector was used for analysis and recording .Zorbax C_{18} column (100cm X 4.5 micron) was used as stationary phase. Mobile phase composed of HPLC grade acetonitrile and methanol (60: 40 v/v) with pH adjusted to 3.8 with O-Phosphoric acid was used. 20µl sample was injected with analysis time or run time of 15 min and flow rate adjusted to 1ml/min. The column was maintained at normal temperature (30° C) and the analytes were observed at 232 nm.

Standard solutions

Simvastatin standard stock solution:

About 80mg of standard drug was transferred into 100ml volumetric flask. Added with few ml of diluent and mixed. The volume was made up to mark with mobile phase to give 800 µg/ml stock solution. From this different concentrations were prepared to give 20-160 µg/ml for construction of calibration curve.

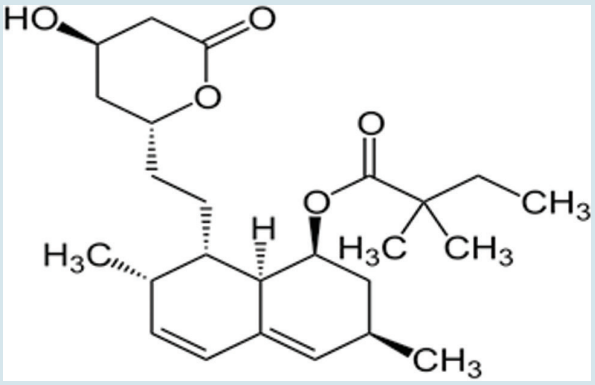

Figure 1: Structure of Simvastatin.

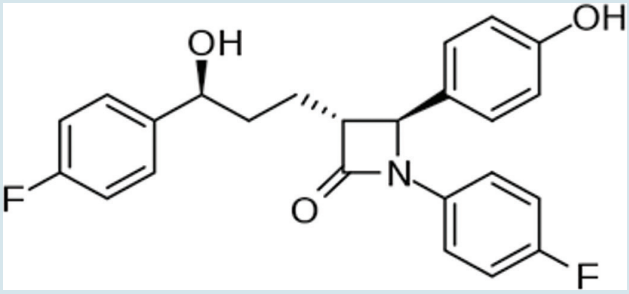

Figure 2: Structure of Ezetimibe.

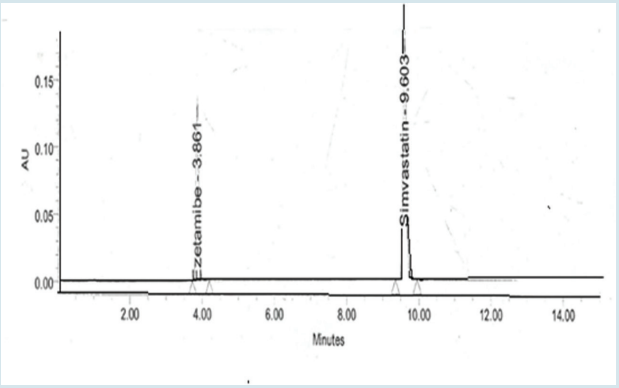

Figure 3: Chromatogram of Standard drug (Simvastatin+Ezetimibe) Sample.

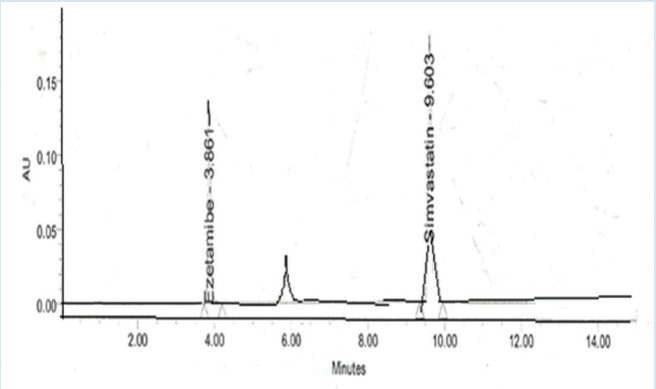

Figure 4: Chromatogram of Marketed Sample.

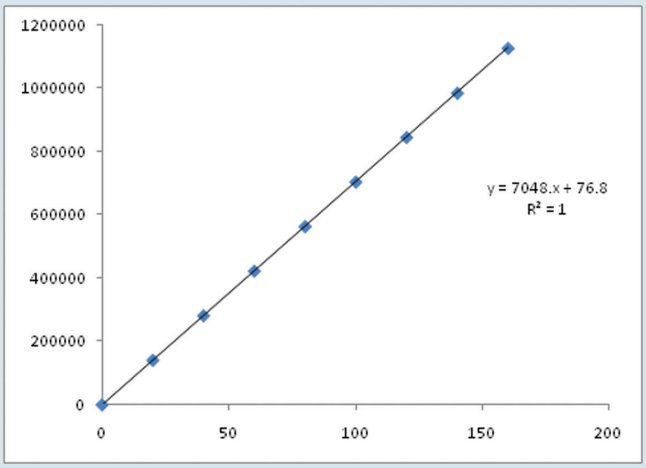

Figure 5: Linearity curve of Simvastatin.

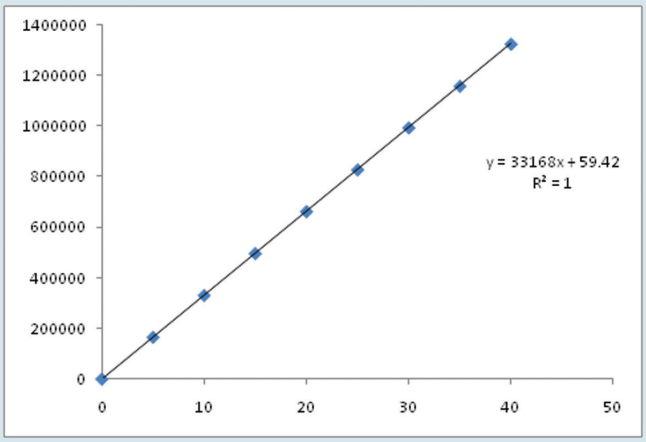

Figure 6: Linearity curve of Ezetimibe.

Ezetimibe standard stock solution:

About 40 mg of ezetimibe was weighed and transferred to a 100 ml volumetric flask. Then sulublized with diluents and vortexed well. The volume was adjusted to the level with mobile phase to give 400 µg/ml. From this different concentrations were prepared to give 5-40 µg/ml for construction of calibration curve.

Standard solution:

Five ml of Ezetamibe standard stock solution and 20 ml of Simvastatin standard stock solution were taken one 100 ml volumetric flask and diluted to volume with diluent and mix followed by making up of volume with mobile phase to give the required concentration for injection in to the RP-HPLC system.

Assay of Marketed formulation:

Twenty tablets, Simvotin-EZ (80 mg of simvastatin & 10 mg of ezetimibe) were weighed and grinded in to powder form. Acqurately weighed quantity powder form equivalent to 80mg of simvastatin and 20 mg of ezetimibe was weighed and taken in a clean volumetric flask. The contents were diluted using diluent to get rid of additives. The contents were mixed thoroughly using Centrifuge at 5000 rpm for 10 min and filtered through 0.45 micron filter. From the filtrate final concentration was prepared in such a manner to give 160 µg/ml of simvastatin and 20 µg/ml of ezetimibe. Finally 20 µl sample was introduced in to RP-HPLC instrumentation and analyzed.

Table 1: Optimization Results

Optimization Parameters	
Mobile Phase	Methanol:Acetonitrile(40:60)
Flow rate	1.0ml/ min
Auto sampler Temperature	5° c
Injection Volume	20µl
Column	C_{18} (Zorbax)
Column Temperature	30° c
Detection wavelength	232nm
Run time	15 min

Table 2: Results for Linearity of Simvastatin and Ezetimibe

Simvastatin Conc. (µg/ml)	Area	Ezetimibe Conc. (µg/ml)	Area
20	141018	5	165885
40	282036	10	331700
60	423054	15	497612
80	564072	20	663543
100	705090	25	829398
120	846005	30	995421
140	986286	35	1160200
160	1128145	40	1327086

RESULTS AND DISCUSSION

The proposed HPLC method consumed very less amount of chemicals and requisites, which made it cost effective and time saving with high reproducibility. This newer method can be used in pharmaceutical quality control and analytical development laborotories. The HPLC peaks of simvastatin and ezetimibe were represented in (Figure 3). The two peaks were well separated from each other with R_t of 9.603 & 3.861 minutes for simvastatin & ezetimibe (Table 1) consecutively.

Validation

Linearity

The developed method was found to be linear in the range of 20 -160 µg/ml for simvastatin & 5- 40 µg/ml for ezetimibe. Each calibration curve sample was observed by injecting to the RP-HPLC system. Linearity graph was plotted by taking concentration (µg/ml)of each drug sample Vs Area under curve(AUC) individually. The intended developed method was measured by its R^2 value and slope intercept value. They are shown by linear regression equations below (Figure 5, 6) and Table 2.

$$Y_{Simvastatin} = 7048.6X+76.8,\ 'R^2'\ value= 1 \ldots\ldots\ldots\ldots (1)$$

$$Y_{Ezetimibe} = 33168\ X+59.422,\ 'R^2'\ value= 1 \ldots\ldots\ldots\ldots (2)$$

These equations were used to assure linearity of the developed method.

Accuracy and Precision

The accuracy of the method was estimated in terms of recovery studies. The recovery studies were done at three concentration levels and the % recovery and %RSD was reported. From the observed values, % recoveries were found to be very accurate and well within the limits (Table 5).

Precision is the reproducibility of the results. The intraday and inter day precision results in terms of % RSDs for simvastatin and ezetimibe was found to be less than 2%. The result for in the day (intraday precision) & within the day (interday precision) of simvastatin was 1.27 and 1.77 and ezetimibe was 1.63 and 1.83 consecutively. The results (Table 3 and 4) agreed with the conformation that the present RP-HPLC method ,found to be accurate and precise as the results obtained are well within the limits.

Specificity of the developed method

The PDA peaks or chromatograms for simvastatin and ezetimibe in pure drug and marketed sample were recorded and analyzed. In the Marketed

Table 3: Results for Intraday precision

Sample	Area of Ezetamibe	%Assay	Area of simvastatin	%Assay
Sample 1	868688	101.59	1173953	101.89
Sample 2	873655	102.31	1179757	102.54
Sample 3	884742	103.96	1190087	100.79
Sample 4	890371	104.48	1201253	101.62
Sample 5	849702	99.69	1157040	100.76
Sample 6	869600	100.2	1184201	99.95
avg	872793	102.03	1181048	101.25
%RSD		1.63		1.27

Table 4: Results for Interday precision

Sample name	Area of Ezetamibe	%Assay	Area of simvastatin	%Assay
Sample 1	868612	101.19	1173859	99.69
Sample 2	869556	101.31	1179625	101.22
Sample 3	884125	102.96	1189945	101.02
Sample 4	890156	102.48	1201198	100.26
Sample 5	849403	99.88	1156198	101.25
Sample 6	854502	100.59	1145152	100.2
avg	869392.3333	101.4	1174329.5	100.6
%RSD	-	1.83	-	1.77

Table 5: Results for Recovery study of Simvastatin and Ezetimibe

Level	%Recovery of Ezetamibe	%Mean recovery	%Recovery of Simvastatin	%Mean recovery
50% sample1	99.80		99.00	
50% sample2	99.81		100.31	
50% sample3	101.71	100.44	99.71	99.67
100% sample1	101.34		99.80	
100% sample2	100.56		99.34	
100% sample3	99.08	99.99	100.24	99.79
150% sample1	100.43		101.46	
150% sample2	101.14		100.37	
150% sample3	99.98	100.52	101.99	101.27

Table 6: Results for Marketed Sample

Tablet (Simvotin-EZ)	Sl.No.	SIM (%)	EZ (%)
	1	99.23	100.1
	2	99.59	100.3
	3	99.99	99.69
	4	99.32	100.3
	5	99.63	100.2
	6	99.21	99.32
Mean		99.495	99.985
%R.S.D		0.302	0.391

sample chromatogram it is noticed by presence of some co-eluting peaks which might be for the presence of other additives in the formulations.The co-eluting peaks however did not have any interference with the main peaks, which conforms the developed RP-HPLC method is specific.

Limit of Detection and Quantification (LOD & LOQ)

The LOD and LOQ can be calculated by using the formulas $3S_a/b$ & $10S_a/b$ respectively. Where S_a stands for standard deviation of intercept and b represents slope of calibration curve. The minimum concentration levels at which the analytes (simvastatin and ezetimibe) can be detected (LOD) and quantified (LOQ) were precisely found to be 1.34 µg/ml, 4.489 µg/ml and 0.253 µg/ml, 0.846 µg/ml respectively.

Assay results for combined dosage form

The % recovery of simvastatin and ezetimibe were found to be 99.495 and 99.985 respectively with %RSD values 0.302 & 0.391, signify that the prescribed assay method is precise and accurate. The results are represented in Table 6 for six replicates (n=6). The chromatogram for the combined dosage form is shown in Figure 4.

CONCLUSION

A new reverse phase liquid chromatographic method indicating assay of simvastatin and ezetimibe simultaneously in bulk and combined tablet dosage form is implemented. The method is simple, reliable, sensitive, accurate, reproducible and cost effective for the successful estimation of both the drugs simultaneously in bulk and tablet dosage form. The method was completely validated with all pertinent documents showing that all results are well within the limits. Also the method was found to be free of interference due to other ingredients or additives used in formulation. Therefore the method is suitable for routine analysis of simvastatin and ezetimibe in bulk and combined tablet dosage form in quality control laboratories.

AUTHOR'S CONTRIBUTIONS

NKS and MS planned and designed the whole work. NKS and AV did the method development and some validation parameters like accuracy and precision studies. MS and BVL did the estimation in marketed formulation. Finally AKM and CKS did rest of the validation parameters.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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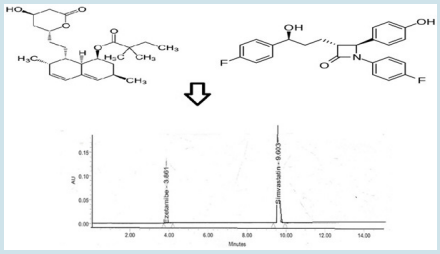
PICTORIAL ABSTRACT



SUMMARY

- A simple, time saving, precise and cost effective reverse phase high performance liquid chromatographic (RP-HPLC) method developement was achieved for the determination and estimation of simvastatin and ezetimibe in its pure form and combined formulation by using mobile phase consisted of acetonitrile and methanol in a ratio of 60:40 (v/v).
- The separation was observed at 232 nm with flow rate adjusted to 1 ml/min. simvastatin and ezetimibe were retained at 9.603 and 3.861 minutes successively.
- Validation was done for the developed method based upon different parameters like linearity, accuracy, precision, limit of detection and limit of quantitation.

ABOUT AUTHORS

Dr. Nalini Kanta Sahoo: Completed his Ph.D in Pharmaceutical analysis from Siksha "O" Anusandhan University, Bhubaneswar. His research area focused on Analytical and bioanalytical chemistry. He published one book and 30 research articles.

Mrs. Madhusmita Sahu: Completed her M. Pharmacy in Pharmacology and recently working in MNR college of pharmacy. Her research area includes various screening methods. She published around 20 research publications.