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Research Article

VALIDATED STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND EZETIMIBE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Simvastatin in combination with Ezetimibe using reverse phase Sunfire C_{18} column(250mm x 4.60mm,5µ) PDA(2998) with UV detector at 225 nm . The mobile phase consisting of ACN, Potassium dihydrogen phosphate (pH 7.2) in the ratio of 60:40 (v/v) and at a flow rate of 1.8 mL/min. The method was linear over the concentration range for Simvastatinin and for Ezetimibe 50-150µg/ml. The % recoveries of active pharmaceutical ingredient (API) Simvastatin and Ezetimibe were found to be in the range of 99 %,100 %. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Simvastatin and Ezetimibe in combined tablet dosage form.

Keywords: Simvastatin, Ezetimibe, HPLC, Validation.

INTRODUCTION

Simvastatin (SIM) is Anticholesteremic Agents, Antilipem Agents, Hydroxymethylglutaryl-CoA, Reductase Inhibitors, Dyslipidemia, Cardiovascular disease, Cholesterolemias. It competitively inhibit HMG co-enzyme A reductase, a rate limiting step in cholesterol synthesis. Reduce cholesterol synthesis results in compensatory increase in uptake of plasma cholesterol mediated by increase in number of LDL receptors. therefore LDL level in plasma reduces. Its chemical name is described as (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-

1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethyl butanoate[1] (Fig. 1). Ezetimibe (EZ) is Anticholesteremic, Insulin Resistance, Gall stones, Dyslipidmiaae, Cholesterol Absorption Inhibitor. Ezetimibe acts within intestine to reduce cholesterol absorption. Cholesterol is absorbed from the small intestine by a process that includes specific transporters that have not been completely characterized. Ezetimibe appears to block one or more of these cholesterol transporters, reducing cholesterol absorption. Its chemical name is described as (3*R*,4*S*)-1-(4-fluorophenyl)- [(3*S*)-3-(4-fluorophenyl)-3hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one[2] (Fig. 2).

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product.

Literature survey revealed few analytical methods is reported for both the drugs in alone. Very few analytical methods have been reported in combination of Ezetimibe and Simvastatin like, Spectrophotometry[3-8], HPLC[9-15] and HPTLC[16-21] methods. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV detection for the analysis of Ezetimibe and Simvastatin in bulk samples and in combined dosage formulation.

EXPERIMENTAL

Chemicals and reagents

The pharmaceutical grade pure samples of amlodipine besylate (99.58%) and metoprolol succinate (99.55%) were received as gift samples from Aurobindo Pharma Ltd., Hyderabad. Amlodipine besylate and metoprolol succinate tablets were purchased from local market. Milli-Q water, HPLC grade acetonitrile and analytical grade Potassium dihydrogen orthophosphate (Merck – HPLC grade) Orthophosphoric acid (Merck – HPLC grade) Ammonium acetate (Merck-GR) Methanol (Merck HPLC grade) Acetonitrile (Merck – HPLC grade) WATER.

Apparatus and chromatographic condition

The chromatographic separation was performed on a HPLC system (WATERS) Series Alliance e2695 Software EMPOWER- 2, integrated with Auto Sampler and 2998 PDA detector. The analytical columns INERTSIL ODS3(250mm,4.6mm,5µ), SUNFIRE C18((250mm,4.6mm,5µ), SYMMETRYC18 (250mm,4.6mm,5µ) of make Bischoff Chromatography was used for the separation. The mobile phase consisted of ACN, Potassium dihydrogen phosphate (pH 7.2) in the ratio of 60:40 (v/v) (6.8 g of Potassium dihydrogen Phosphate was dissolved in 1000ml of milliQ water. Adjusted the pH to 7.2 with Triethylamine). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.8 mL/min and the detector wavelength was set at 225 nm. The injection volume was 15 µL. The methanol was used as diluent.

Preparation of Ezetimibe and Simvastatin standard & sample solution

Standard solution preparation

Accurately weigh and transfer 10 mg of amlodipine and 50 mg of metoprolol working standard into a 50 mL clean dry volumetric flask add about 50 mL of methanol and sonicate to dissolve it completely and make volume up to the mark with methanol. Made up to the mark with the methanol to get a concentration of 100µg/ml**.** It was degassed in ultrasonicator and then filtered through membrane filter of 0.45µ pore size.

Sample Solution Preparation

Weigh and finely powder not fewer than 10 tablets. Accurately weigh and transfer sample equivalent to 10 mg of Ezetimibe and Simvastatin into a 100 mL clean dry volumetric flask add about 75 mL of methanol and sonicate to dissolve it completely and make volume up to the mark with and made upto the mark with methanol to get the concentration of 100µg/ml solution. The solution was degassed and filtered through membrane filter of pore size 0.45µ.

Procedure

Inject 15µL of the standard, sample solution into the chromatographic system and measure the peak areas for amlodipine and metoprolol and calculate the % assay value.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to

ICH guidelines[12]. Obtained validation parameters are presented in Table 1.

Linearity

The linearity for HPLC method was determined at five concentration levels ranging from 50 - 150 µg/mL for Simvastatin and 50 - 150 µg/mL for Ezetimibe**.** The calibration curve was constructed by plotting response factor against respective concentration of Simvastatin and Ezetimibe. The plots of peak area Vs respective concentration of Simvastatin and Ezetimibe were found to be linear in the range of 10-50 µg/mL and 40-200 µg/mL with coefficient of correlation (r^2) 0.999 and 0.999 for Simvastatin and Ezetimibe respectively. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Simvastatin and Ezetimibe were given in Fig. 3 and Fig. 4.

Recovery

Three different samples of known concentration ranging from 10-50 µg/mL for Simvastatin and 40-200 µg/mL for Ezetimibe were prepared and these are analyzed against standard solution**.** The mean recoveries of both the drugs were found to be 99.67%, 100.33% respectively. The obtained results are presented in Table 2.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to 0.29µg/ml and 0.97µg/ml for Ezetimibe and 0.61µg/ml and 2.05µg/ml for Simvastatin. The LOD and LOQ showed that the method is sensitive for Simvastatin and Ezetimibe.

System suitability test

The specificity of this method was determined by complete separation of Simvastatin and Ezetimibe as shown in Fig. 5 with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of Simvastatin and Ezetimibe was less than 2% and resolution was satisfactory. The average retention time for Simvastatin and Ezetimibe were found to be 2.35 and 7.23 respectively, for five replicates. The peaks obtained for Simvastatin and Ezetimibe were sharp and have clear baseline separation. Analysis was also performed for active Simvastatin and Ezetimibe, placebo sample (All the ingredients except active Simvastatin and Ezetimibe) both at stressed and unstressed condition. After analysis it was found that there is no interference of peak in the amlodipine and metoprolol region for the stressed, placebo & active sample. Hence the developed method was specific for the analysis of this product**.**

Precision

The method precision study was performed for five sample preparations of marketed formulations. A study was carried out for intermediate precision with the same analyst on the different day for five sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase P^H and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The Intra-day and Inter-day precision results are presented in Table 3. The assay results of tablet dosage formulation by the proposed method are presented in Table 4.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Simvastatin and Ezetimibe remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 5.

Control sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 10 mg of Simvastatin and 10 mg Ezetimibe into a 100 mL clean dry volumetric flask, add about 75 mL of methanol and sonicate to dissolve it completely and make volume up to the mark with the diluent. Filter the solution through 0.45µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL

volumetric flask and dilute up to the mark with diluent.

Acid degradation sample
Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask, add about 10 mL of 0.1N acid (Hydrochloric acid), refluxed for 30 minutes at 60°C, then cooled to room temperature, neutralize with 10 ml of 0.1N base (Sodium hydroxide) and make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of acid degradation was given in Fig. 6.

Base degradation sample
Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of 0.1N base (Sodium hydroxide), refluxed for 30 minutes at 60°C, then cooled to room temperature, neutralize with 10 ml of 0.1N acid (hydrochloric acid) and make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of base degradation was given in Fig. 7.

Peroxide degradation sample
Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of 1% H_2O_2, refluxed for 30minutes at 60°C, then cooled to room temperature, make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of oxidative degradation was given in Fig. 8.

Water degradation sample
Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of H_2O, refluxed for 30minutes at 60°C, then cooled to room temperature, make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of oxidative degradation was given in Fig. 9.

Thermal degradation sample
Weigh and finely powder not fewer than 2 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask. Add about 75 mL of methanol and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of thermal degradation was given in Fig. 10.

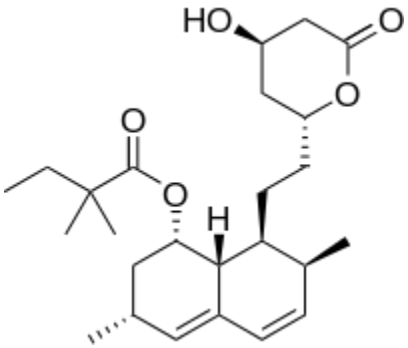

Fig. 1: Chemical structure of Simvastatin

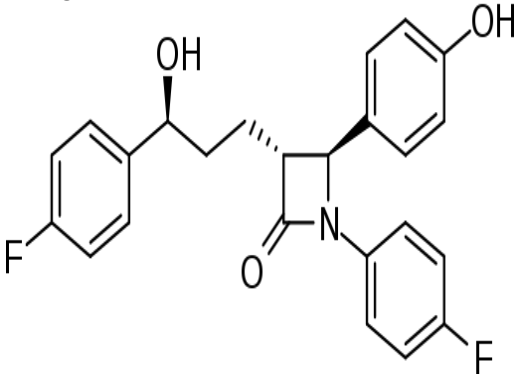

Fig. 2: Chemical structure of Ezetimibe

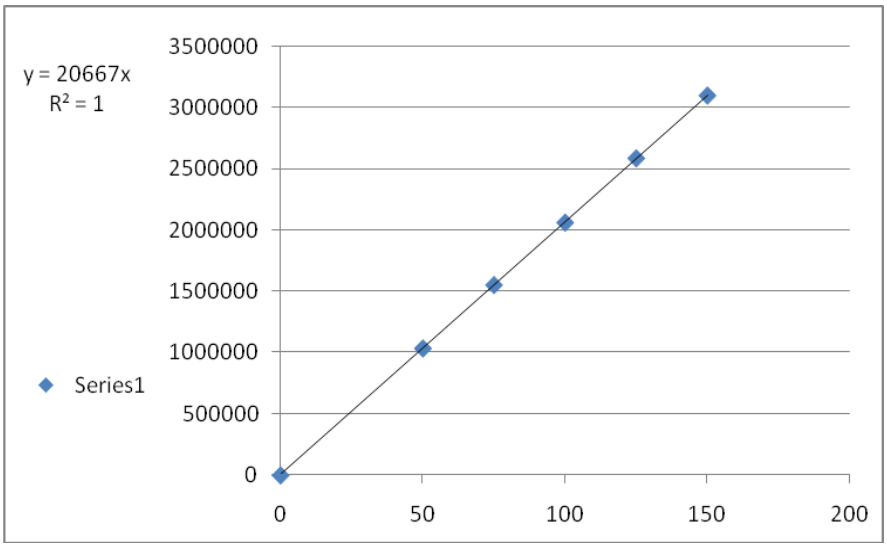

Fig. 3: Calibration curve for Ezetimibe

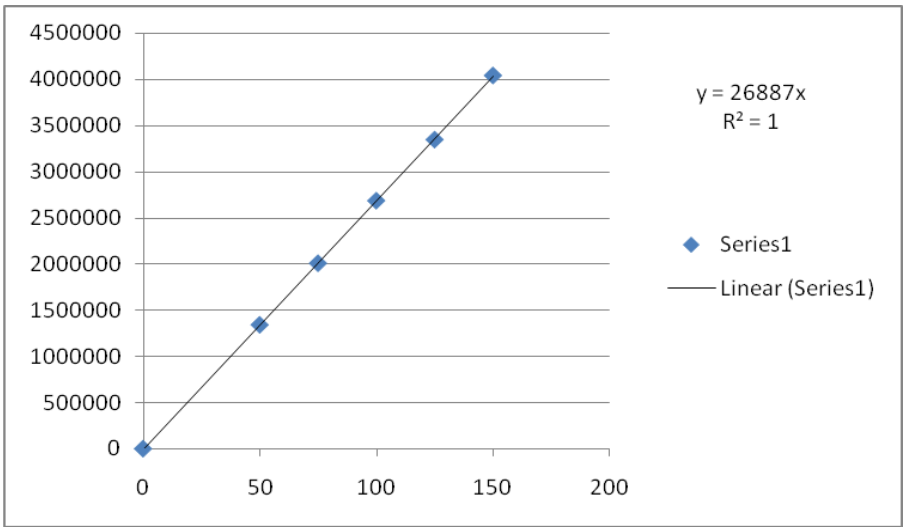

Fig. 4: Calibration curve for Simvastatin

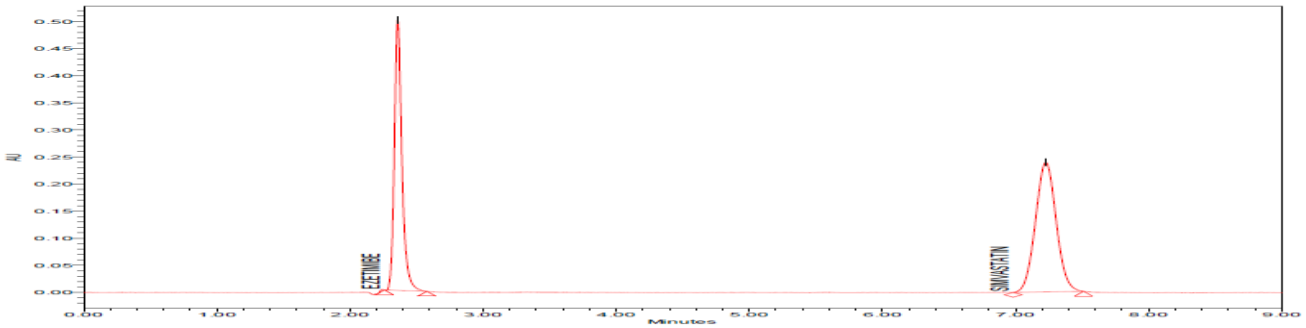

Fig. 5: Typical chromatogram of Ezetimibe and Simvastatin

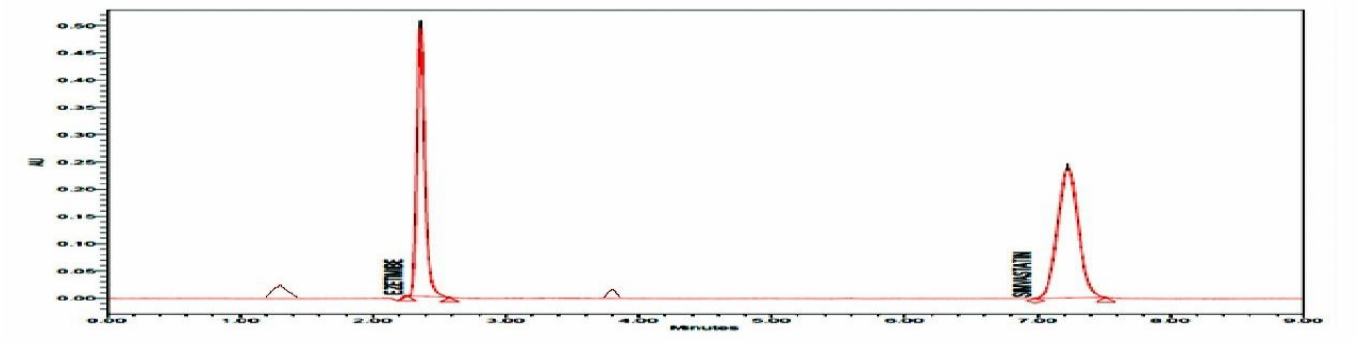

Fig. 6: Acid degradation chromatogram of Ezetimibe and Simvastatin

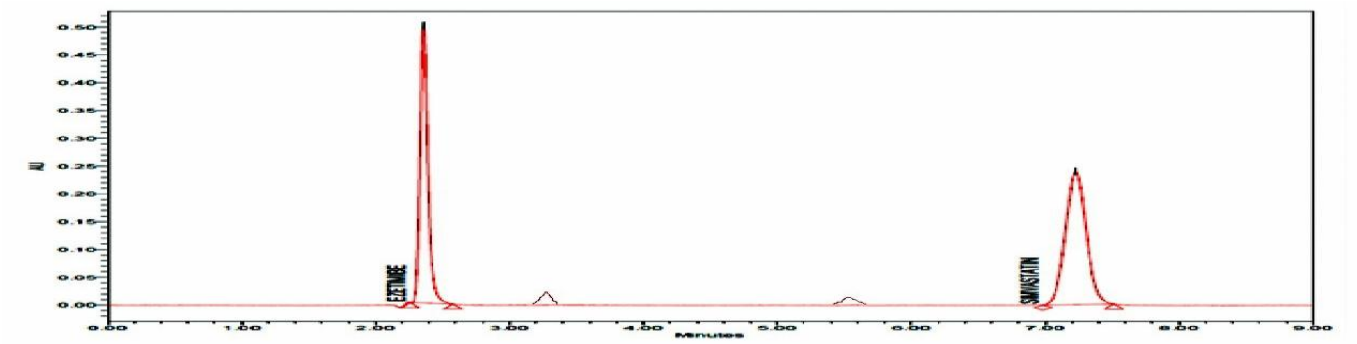

Fig. 7: Base degradation chromatogram of Ezetimibe and Simvastatin

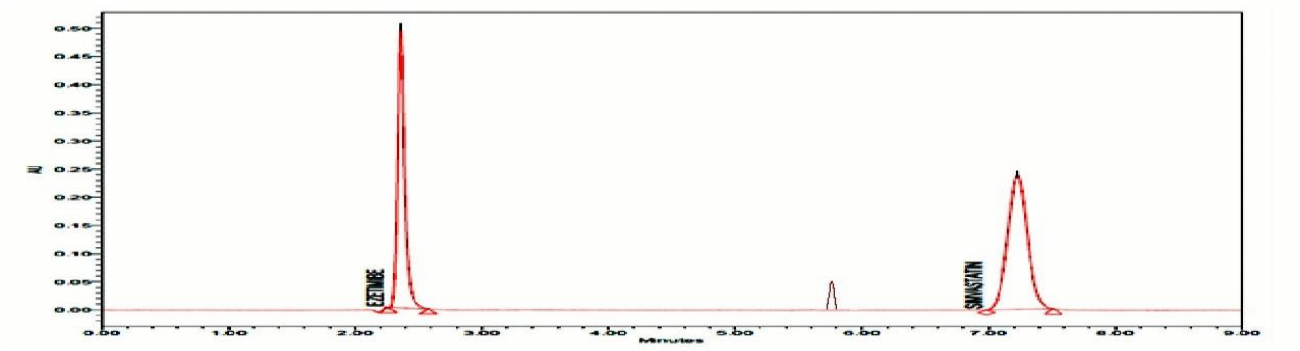

Fig. 8: Peroxide degradation chromatogram of Ezetimibe and Simvastatin

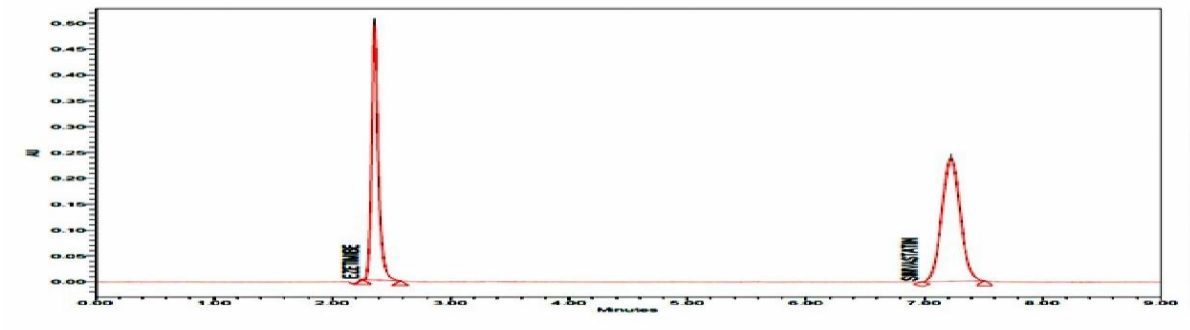

Fig. 9: Water degradation chromatogram of Ezetimibe and Simvastatin

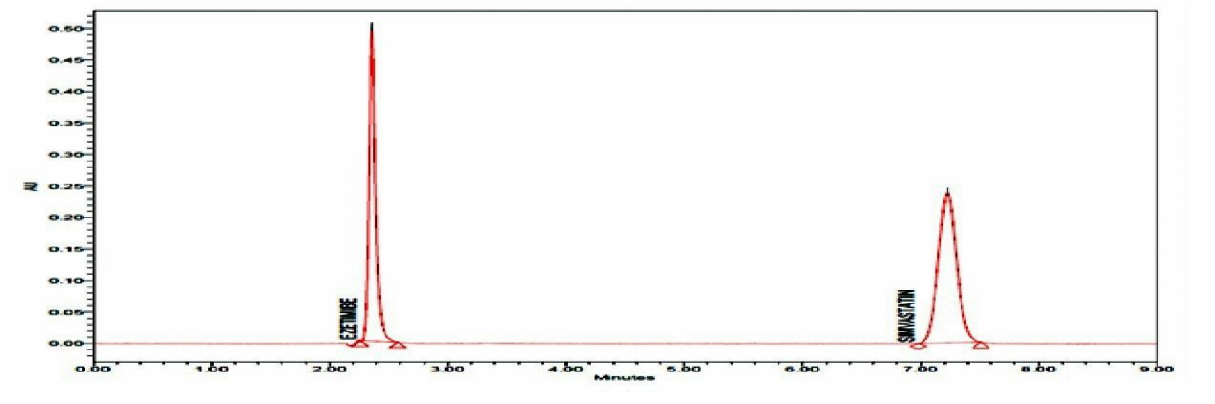

Fig. 10: Thermal degradation chromatogram of Ezetimibe and Simvastatin

Table 1: Analytical validation parameters (System suitability and Linearity)

Parameter	Ezetimibe	Simvastatin
Linearity	50 - 150 µg/mL	50 - 150 µg/mL
Slope	66296.0	10702.0
Intercept	13194.0	115866.0
% Y-Intercept	19.9	1082.7
Residual Sum of Squares	17845.0	45466.0
CC(r)	1	1
RSQ(r^2)	1	1
LOD	0.29	0.61
LOQ	0.97	2.05
Theoretical Plates	8683	10051
Tailing Factor	1.3	1.0
Retention Time (min)	2.35	7.23

Table 2: Recovery studies of Ezetimibe and Simvastatin

Recovery data of Ezetimibe					
Concentration (at specification level)	**Peak Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean % Recovery**
50%	1045173	49.500	50.08	101	99.66
100%	2046693	99	98.18	99	
150%	3052858	148.500	146.78	99	
Recovery data of Simvastatin					
Concentration (at specification level)	**Peak Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean % Recovery**
50%	1332677	49.00	49.11	100	100.3
100%	2659682	98	98.02	100	
150%	4045014	147.00	148.90	101	

Table 3: Intra-day and Inter-day precision of Ezetimibe and Simvastatin

Drug	Sample Weight(mg)	Intra-day precision		Inter-day precision	
		SD	%RSD	SD	%RSD
Ezetimibe	172.8	99.94	0.62	99.92	0.6
Simvastatin	172.8	98.87	0.81	98.97	0.8

Table 4: Assay result of tablet dosage formulation

Drug	Label strength (mg)	Amount found (mg)	% Assay
Ezetimibe	10	9.947	99.47%
Simvastatin	10	9.99	99.97

Table 5: Forced degradation studies of Ezetimibe and Simvastatin

Stress Conditions	Degradation Time	Peak Area		% Degradation		% of Active drug present after degradation	
		Ezetimibe	Simvastatin	Ezetimibe	Simvastatin	Ezetimibe	Simvastatin
Control	-	2087273	2696175	-	-	-	-
Acid	1 hour	2000235	2121378	3.08	3.44	96.92	96.56
Base	1 hour	2001354	2212143	4.46	4.48	95.54	95.52
Peroxide	1 hour	2001187	2122465	5.46	7.74	94.54	92.26
Water		2001298	2131764	3.11	3.64	96.89	96.36
Thermal	48 hours	2001265	2123476	3.76	3.22	96.24	96.78

CONCLUSION

The findings of the present investigation are summarized as follows

1. A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc.
2. In the present method a Sunfire C_{18} (250×4.6mm I.D,5µ) column has been used for Ezetimibe & Simvastatin drugs respectively.
3. Mobile phase used was Acetonitrile: Phosohate buffer (60:40%v/v) for drugs Ezetimibe & Simvastatin respectively, Retention of Ezetimibe & Simvastatin has more dependence on the mobile phase.
4. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases. Ezetimibe & Simvastatin were eluted at acceptable retention times and got good resolution.
5. Several assay methods has been developed for the determination of Ezetimibe & Simvastatin in formulations and biological fluids but this method is most economic and accurate so this method is very useful for the determination of Ezetimibe & Simvastatin in tablet formulations . This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of drug Ezetimibe & Simvastatin in tablet formulation.

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