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METHOD DEVELOPMENT AND VALIDATION OF EZETIMIBE AND SIMVASTATIN IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Lornoxicam and Thiocolchicoside in tablets by reverse phase C8 column (X terra, 4.6 x 250mm, 5µm, Make: ACE) or equivalent. The sample was analyzed using Buffer (Weighed 2.5milligrams of Sodium di hydrogen ortho phosphate in 1000 ml HPLC water, adjust pH 4.0 with sodium hydroxide) Acetonitrile in the form of 70% and 30% as a mobile phase at a flow rate: 1.0 mL per min and detection at 230 nm. The retention time for Simvastatin and Ezetimbe was found to be 4.65and 6.80 min respectively. The limit of detection is 0.37 µg/ml and the limit of quantitation is 0.12 µg/ml. Linearity for Lornoxicam and Thiocholchicoside were found in the range of 1-50 µg/ml for SIM &5 - 25 µg/ml for EZE. The Accuracy % recoveries are between 98.0 % to 102.0% .The present method is successfully applied for the estimation of Lornoxicam market formulation-Tablet.

Keywords: Simvastatin, Ezetimbe, Reverse-Phase High-Performance Liquid chromatography.

INTRODUCTION

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Ezetimibe and Simvastatin in pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. The literature review reveals that there are some analytical methods reported for Ezetimibe and Simavastatin either individually or in combination with other drugs by RP-HPLC method and most of the work done on biological fluids. Various analytical methods like UV, HPLC, TLC, HPTLC, LC-MS-MS, are reported for the analysis of these two compounds individually and also in combination with other drugs. Present study aims to develop simple, rapid, greater sensitivity and faster elution by RP-HPLC for the simultaneous estimation of Ezetimibe and Simvastatin and to decrease retention time, low cost. The developed method will be validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines [1-4].

Instruments and Equipment's Used

Instruments

- UV-3000$^+$ LABINDIA Double beam with UV win 5 software UV-Visible spectrophotometer with 1cm matched quartz cells.
- WATERS HPLC, Model: Aliance 2695, UV- Visible Dual absorbance Detector 2487, with an automated sample injector. The output signal was monitored and integrated using Empower 2 software, Symmetry C8 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent column was used for separations.

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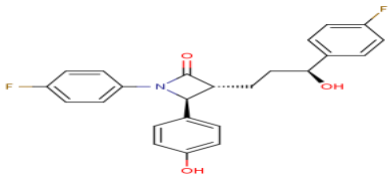
DRUG PROFILE

a) Ezetzamibe:

Structure:



Chemical name :(3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3- hydroxypropyl]-4- (4-hydroxyphenyl)azetidin-2-one

Molecular formula : $C_{24}H_{21}F_2NO_3$

Molecular Weight : 409.4252

Half life : 19–30 hours

Dose : 10 mg

Description : white coloured CRYSTALLINE powder

Solubility : freely to very soluble in ethanol, methanol, acetone.

Melting point : 164–166 °C

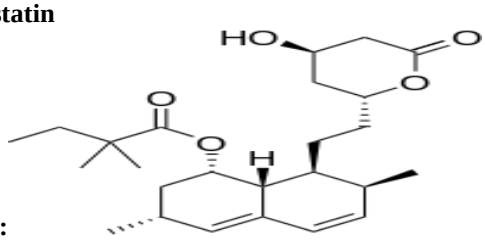
Category : Anticholesteremic Agents, Cholesterol Absorption Inhibitors

Uses : inhibits the absorption of cholesterol from the intestine

Side effects : gastrointestinal disturbances, headache, fatigue, myalgia; rarely arthralgia, hypersensitivity reactions (including rash, pancreatitis, cholelithiasis, cholecystitis, thrombocytopenia, myopathy, and rhabdomyolysis

Brand Name : Zedoc, Ezetib, Ezetrol, Maxetibe, Zemitra, Zetavim

b) Simvastatin



Structure:

Chemical name :((1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1yl2,2dimethylbutanoate.

Molecular formula : $C_{25}H_{38}O_5$

Molecular Weight : 418.5662

Half life : 3 hours

Category : Anticholesteremic Agents, Antilipidemic Agents,

Dose : 5 mg to 80 mg

Description : white coloured powder

Solubility : ethanol, Soluble in DMSO,

Storage conditions : Store at 20°C.

Use : Treatment of dyslipidemia and the prevention of cardiovascular disease.

Brand Name : Cholestat, Coledis, Colemin, Lipex, Labistatin [5-9].

Reagents and Standard – Ezetimibe & Simvastatin Tablets:

a. Water HPLC Grade.

b. Ezetimibe & Simvastatin Working Standards

c. Acetonitrile HPLC Grade

d. Ortho phosphoric acid

Preparation of Phosphate buffer:

Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 4 with Orthophosporic acid.

Preparation of mobile phase

Mix a mixture of above buffer 500 mL (50%) and 500 mL of Acetonitrile HPLC (50%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluent.

Preparation of the Ezetimibe & Simvastatin Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Ezetimibe and Simvastatin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution).

Further pipette 5ml of Ezetimibe & Simvastatin the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3ml of Ezetimibe & Simvastatin the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 10 mg of Ezetimibe and Simvastatin sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution).

Further pipette 5ml of Ezetimibe & Simvastatin of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Further pipette 3ml of Ezetimibe & Simvastatin the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the

Ezetimibe and Simvastatin peaks and calculate the %Assay by using the formulae [10-14].

System Suitability

Tailing factor for the peaks due to Ezetimibe & Simvastatin in Standard solution should not be more than 1.5.

Theoretical plates for the Ezetimibe & Simvastatin peaks in Standard solution should not be less than 2000 [15-17].

Calculation: (For Ezetimibe)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation.
As = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = LABEL CLAIM OF Ezetimibe mg/ml.

Table 1. Equipment's

S. No.	Equipment's	Software	Model	Company
1	Electronic Balance	NA	ER200A	Ascoset
2	Ultra-Sonicator	NA	SE60US	Enertech
3	Heating Mantle	NA	BTI	Bio Technics India
4	Thermal oven	NA	---------	Narang
5	pH Meter	NA	AD102U	Adwa
6	Filter Paper 0.45 microns	NA	---------	Milli Pore

Table 2. Materials and Chemicals

S. No.	Chemicals/standards and reagents	Grade	Company
1	Potassium di- Hydrogen Ortho Phosphate	AR	Finar
2	Ortho-Phosphoric Acid	AR	Finar
3	Acetonitrile	HPLC	Merck
4	Water	HPLC	Loba Chemi
5	Ezetimibe	NA	Dr. Reddy's
6	Simvastatin	NA	Dr. Reddy's

Table 3. Optimized Method Parameters

Parameters	Method	
Column(Stationary Phase)	Symmetry C8 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent	
Mobile Phase	pH 4.0 Potassium dihydrogen Phosphate: ACN(50:50)	
Flow rate (ml/min)	0.8 mL per min	
Run time (min)	10 min	
Column temperature(°C)	Ambient	
Volume of injection loop (µl)	20 µl	
Detection wavelength (nm)	236 nm	
Drug RT (min)	**Ezetimibe**	**Simvastatin**
	4.4	8.4
Linearity range (µg/ml)	10-50	10-50
Regression equation	**Ezetimibe**	**Simvastatin**
Correlation coefficient	0.9997	0.9998

Table 4. Assay: Analysis of Commercial Formulation

Formulation	Labeled Amount (mg)		% Recovery by proposed method		%RSD	
	Ezetimibe	Simvastatin	Ezetimibe	Simvastatin	Ezetimibe	Simvastatin
	10	10	99.5	99.7	0.01	0.02

Table 5. Typical chromatogram of formulation (30µg/ml of Simvastatin, 30µg/ml of Ezetimibe)

Accuracy

% of pure	Pure drug		Formulation		Ezetimibe		Simvastatin	
	Ezetimibe	Simvastatin	Ezetimibe	Simvastatin	%	Statistical	%recovery	Statistical

drug spiked					recovery	analysis		analysis
50%	15	15	30	30	99.2	Mean = 99.10 SD = 0.135 %RSD = 0.13	99.3	Mean = 98.05 SD = 0.058 %RSD = 0.05
50%	15	15	30	30	99.1		99.0	
50%	15	15	30	30	99.1		99.5	
100%	15	15	30	30	99.6	Mean = 97.58 SD = 0.032 %RSD = 0.03	99.4	Mean = 98.03 SD = 0.045 %RSD = 0.04
100%	15	15	30	30	99.6		99.5	
100%	15	15	30	30	99.5		99.8	
150%	15	15	30	30	99.0	Mean = 98.80 SD = 0.005 %RSD = 0.005	99.5	Mean = 98.03 SD = 0.015 %RSD = 0.01
150%	15	15	30	30	99.3		99.3	
150%	15	15	30	30	99.5		99.5	

Table 6. Typical chromatograms of Standard Drugs Ezetimibe and Simvastatin + Ezetimibe

S.No	Linearity Level	Concentration	Area
1	I	10ppm	1030282
2	II	20ppm	1958485
3	III	30ppm	2935948
4	IV	40ppm	3876589
5	V	50ppm	4899632
Correlation Coefficient			0.9997

Table 7. Simvastatin

S.No	Linearity Level	Concentration	Area
1	I	10ppm	1267866
2	II	20ppm	2410930
3	III	30ppm	3614299
4	IV	40ppm	4793817
5	V	50ppm	6044368
Correlation Coefficient			0.9998

Precision:

Table 8. System Precision (Intra Day)

The results are summarized **EZETIMIBE**

Injection	Area
Injection-1	2876374
Injection-2	2912457
Injection-3	2899301
Injection-4	2910649
Injection-5	2909773
Average	2901711
Standard Deviation	15067.5
%RSD	0.52

The results are summarized SIMVASTATIN

Injection	Area
Injection-1	3570678
Injection-2	3605812

Injection-3	3590517
Injection-4	3599152
Injection-5	3596150
Average	3592462
Standard Deviation	13368.8
%RSD	0.37

The results are summarized EZETIMIBE

Injection	**Area**
Injection-1	2914140
Injection-2	2910592
Injection-3	2910747
Injection-4	2913191
Injection-5	2919374
Average	2913609
Standard Deviation	3570.2
%RSD	0.12

The results are summarized SIMVASTATIN

Injection	**Area**
Injection-1	3597821
Injection-2	3591676
Injection-3	3580581
Injection-4	3592387
Injection-5	3603865
Average	3593266
Standard Deviation	8621.2
%RSD	0.24

Table 9. Robustness Ezetimibe

S.No	**Flow Rate (ml/min)**	**Area**	**%RSD**	**System Suitability Results**	
				Plate Count	**Tailing**
1	**Less flow 0.6**	3328442	0.016	4537	1.3
		3345692			
		3378545			
2	**Actual flow 0.8**	2910592	0.830	4590	1.3
		2910747			
		2913191			
3	**More flow 1.0**	2585587	0.3165	4264	1.3
		2567893			
		2597432			

Simvastatin

S.No	**Flow Rate (ml/min)**	**Area**	**%RSD**	**System Suitability Results**	
				plate count	**tailing**
1	**Less flow 0.6**	4109709	0.464	7869	1.1
		4108935			
		4106734			
2	**Actual flow 0.8**	3591676	0.340	7822	1.1
		3580581			
		3592387			
3	**More flow 1.0**	3192667	0.076	7232	1.1
		3187974			
		3184739			

Ezetimibe

S.No	Mobile Phase	Area	%RSD	System Suitability Results	
				Plate Count	Tailing
1	**Less Org**	2891001	0.149	2013	1.7
		2894647			
		2894656			
2	**Normal**	2910592	0.830	4590	1.3
		2910747			
		2913191			
3	**More Org**	2923861	0.292	2148	1.7
		2923464			
		2945737			

Simvastatin

S.No	Mobile phase	Area	%RSD	System Suitability Results	
				plate Count	Tailing
1	Less Org	3580370	0.07	5332	1.2
		3589137			
		3570171			
2	Normal	3591676	0.830	7822	1.1
		3580581			
		3592387			
3	More Org	3590821	0.521	4126	1.3
		3598093			
		3598402			

Fig. 1. Typical chromatogram of Standard (30µg/ml of SIMVASTATIN, 30µg/ml of EZETIMIBE)

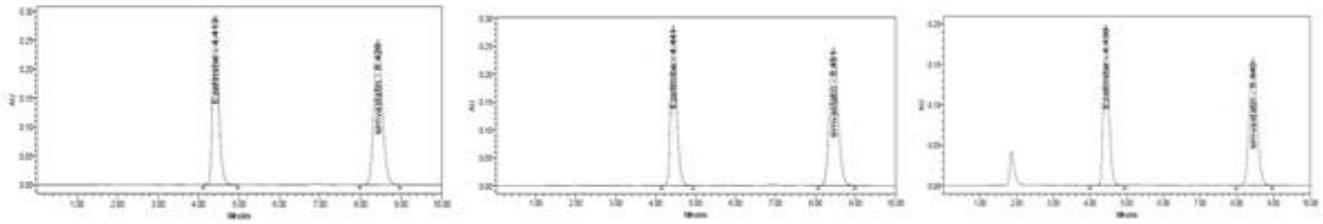


Fig. 2. Typical chromatogram for Accuracy 50 %(EZETIMIBE 45µg/ml & SIMVASTATIN 45µg/ml)

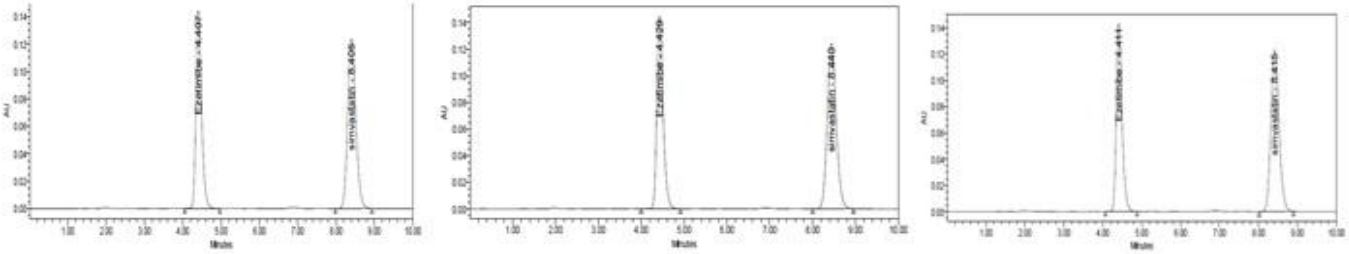


Fig. 3. Typical chromatogram for Accuracy 100 %(EZETIMIBE 60µg/ml & SIMVASTATIN 60µg/ml)

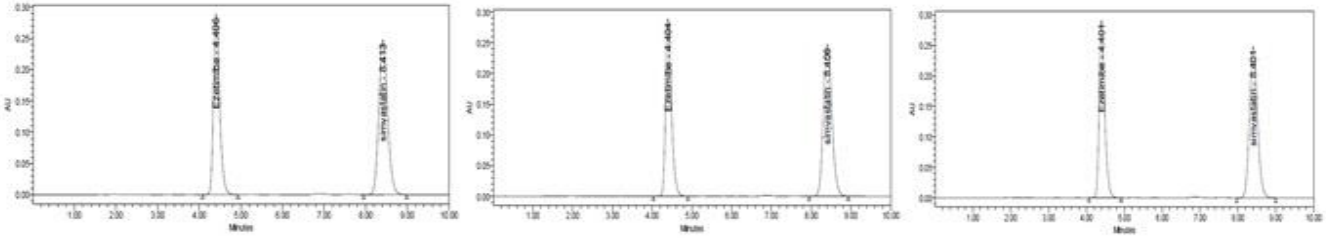


Fig. 4. Typical chromatogram for Accuracy 150 %(EZETIMIBE 75µg/ml & SIMVASTATIN 75µg/ml)

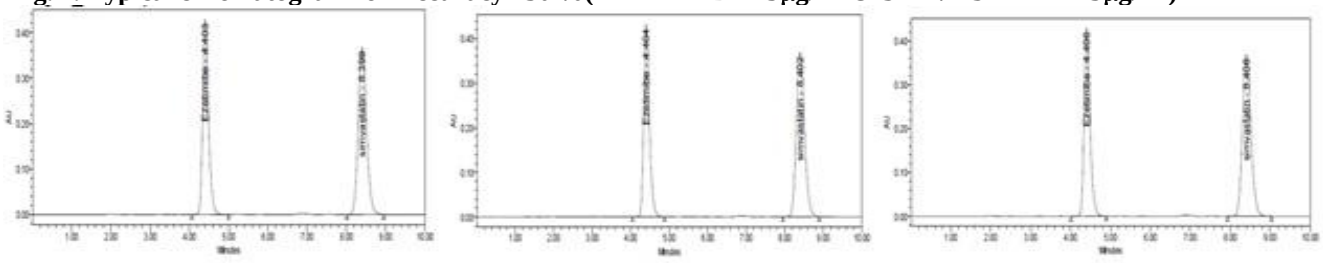


Fig. 5. Linearity:

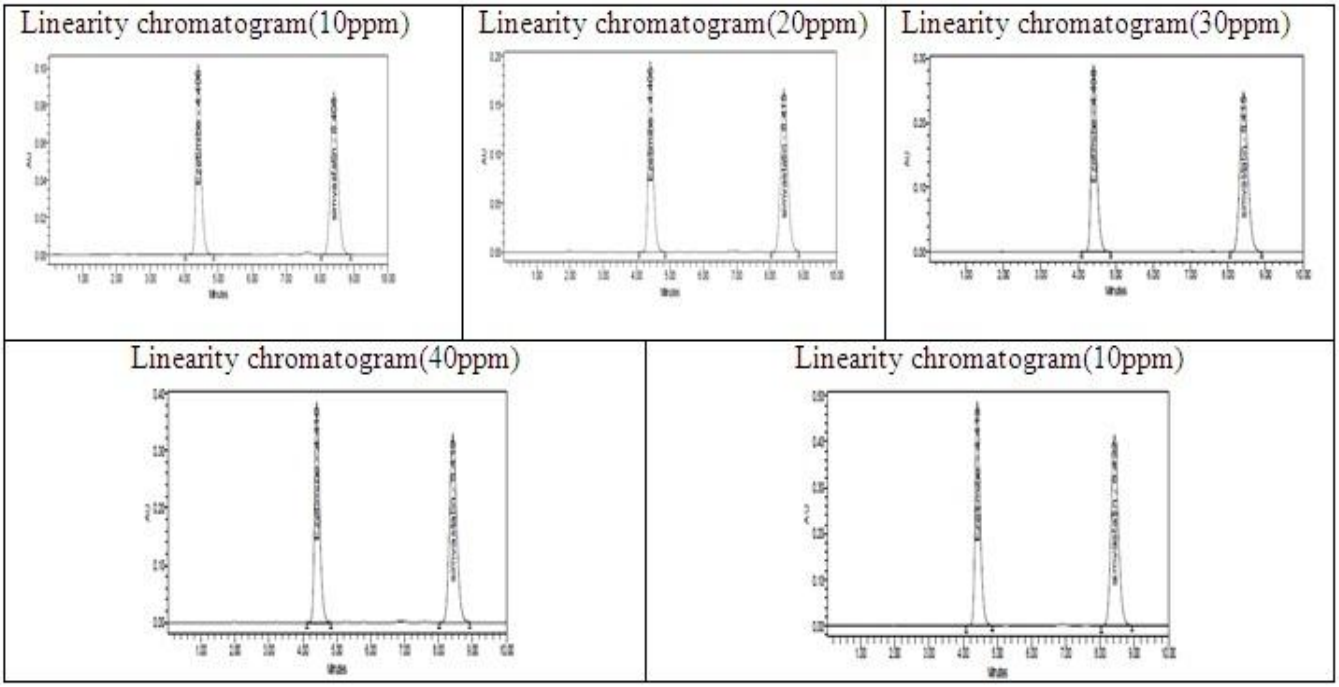


Fig. 6. Linearity plot of EZETIMIBE

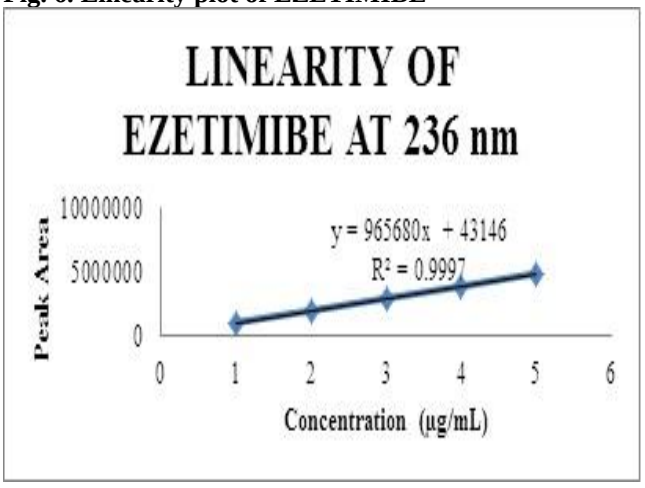


Fig. 7. Linearity plot of SIMVASTATIN

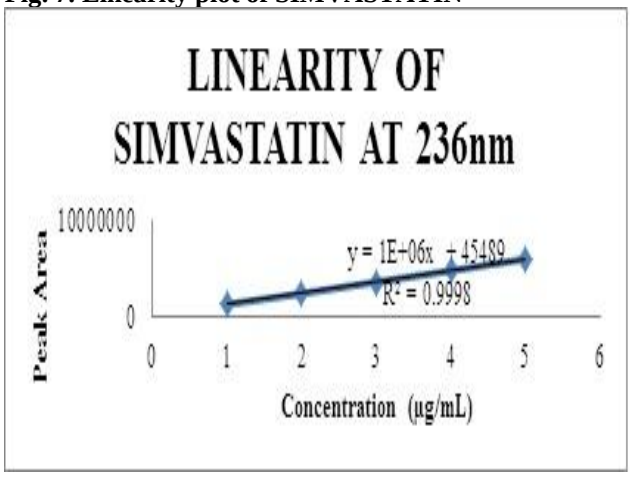


Fig. 8. Typical chromatogram for the System Precision studies (Intra Day) (30µg/ml of SIMVASTATIN, 30µg/ml of EZETIMIBE) System Precision (Inter Day)

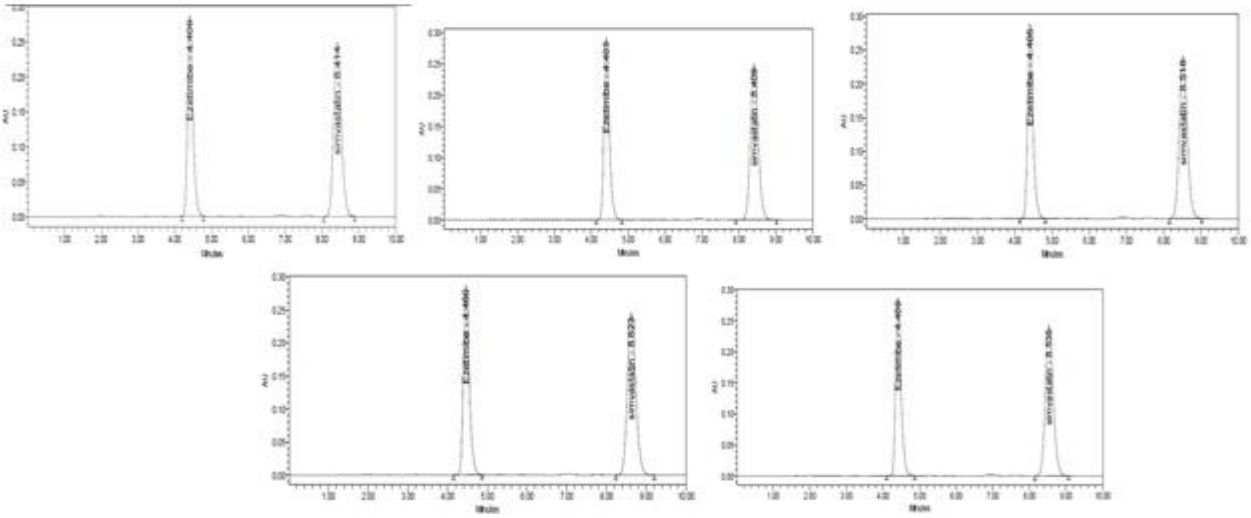


Fig. 9. Typical chromatogram for the System Precision study (Inter Day) (30µg/ml of SIMVASTATIN, 30µg/ml of EZETIMIBE)

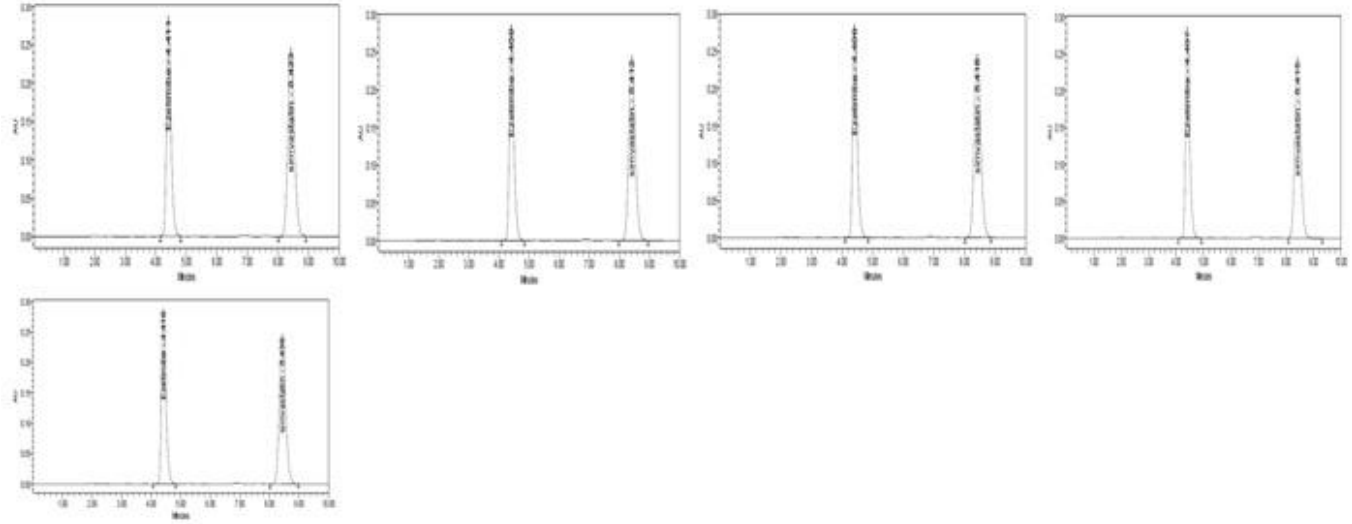


Fig. 10. Robustness Flow

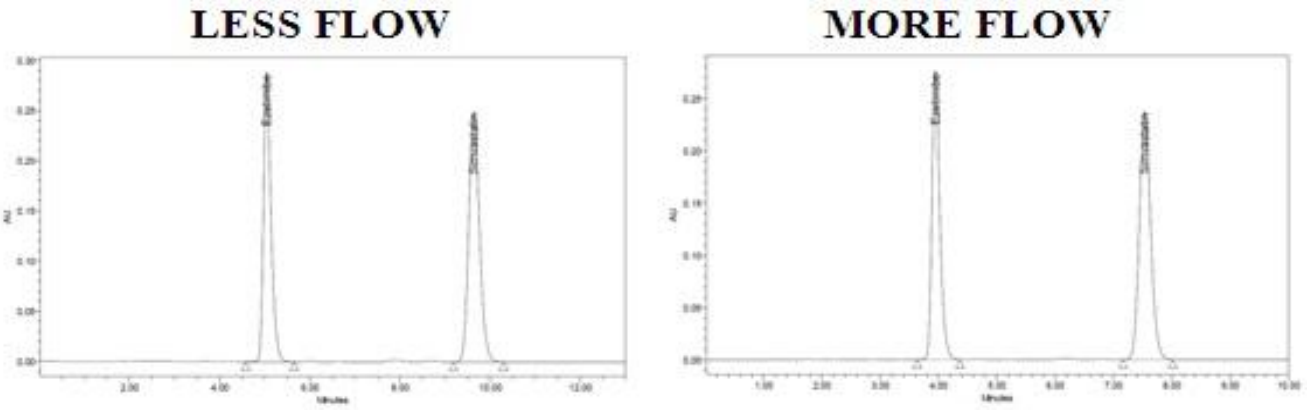
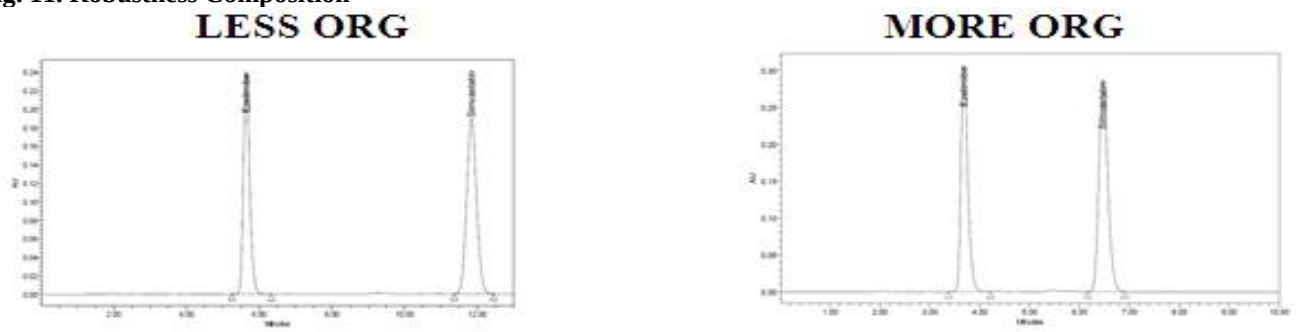


Fig. 11. Robustness Composition



RESULTS

System Suitability Results

Tailing factor Obtained from the standard injection is 1.1. Theoretical Plates Obtained from the standard injection is 7822.

CONCLUSION

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Ezetimibe and Simvastatin in pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims.

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