INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.7 | Issue 1 | Jan - Mar -2018

Journal Home page: www.ijpar.com

Research article *Open Access*

RP-HPLC method for simultaneous estimation of simvastatin and ezetimibe in tablet dosage forms

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ABSTRACT

A rapid, precise, specific, accurate and robust simple RP-HPLC method for the simultaneous determination of Simvastatin and Ezetimibe has been developed and validated. The Stationary phase (column) is Inertsil-ODS C_{18} (250 x 4.6 mm, 5 µ) and Mobile Phase is Methanol: Acetonitrile (80:20), with flow rate of 1.0ml/min using UV detection at 240 nm. The retention time if simvastatin and ezetimibe is 3.125 and 3.380 respectively. The Method shows linearity in the range of simvastatin and ezetimibe with correlation coefficient of 0.9999 for both. The limit of detection (LOD) and limit of quantification (LOQ) for simvastatin and ezetimibe were found to be 0.22 and 0.25 respectively. The % recovery for simvastatin and ezetimibe were found within the range of 99.83 and 99.55 respectively. The developed RP-HPLC method was innovation, suitable for detecting both simvastatin and ezetimibe in tablet dosage form.

Keywords: RP-HPLC, Simultaneous estimation, Simvastatin, Ezetimibe and Tablet dosagre form.

INTRODUCTION

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer's law, which establishes the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer [1-4].

High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a

result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially. The essential equipment consists of an eluent, reservoir, a high-pressure pump, and an injector for introducing the sample, a column containing the stationary phase, a detector and recorder. The development of highly efficient micro particulate bonded phases has increased the versatility of the technique and has greatly improved the analysis of multi component mixtures.

MATERIALS AND METHODS

Preparation of Standard Solution

Weigh down 10mg's of Simvastatin and Ezetimibe drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Chromatographic Conditions

Flow rate : 1.0ml/min
Column : Inertsil - C18, BDS column
Detector wavelength: 240nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 3.125min for SMVSTATN and 3.380 for EZTMBE.

Optimized Method

Mobile Phase: Degassed Acetonitrile and Buffer in the ratio of 60:40 V/V.

Preparation of stock solution

Reference solution: The solution was prepared by dissolving 25.0 mg of accurately weighed Simvastatin and 25.0 mg Ezetimibe in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Simvastatin and Ezetimibe above, sonicated and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 40 mg Simvastatin and 40 mg Ezetimibe was weighed and dissolved in the 70 mL mobile phase with the aid of ultrasonication for 20 min. The content was diluted to 100 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 100.0 mL volumetric flask to give a test solution containing 40 µg/mL Simvastatin and 40 µg/mL Ezetimibe [5].

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Acetonitrile(80:20)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	8 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	240nm
Drug RT (min)	2.7min for SMVSTATN and 3.4 for EZTMBE.

Simvastatin

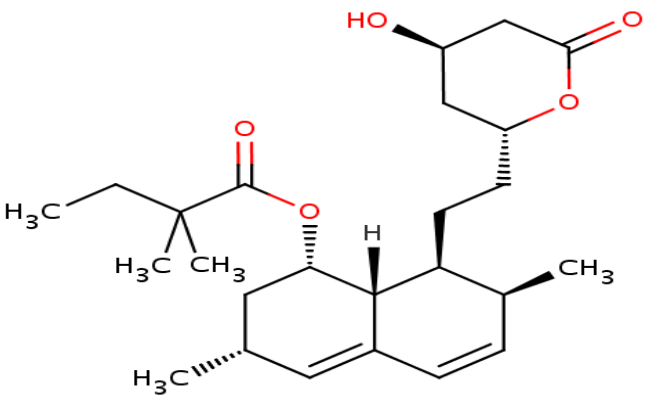

Figure 1. Structure of Simvastatin

Chemical Formula : $C_{25}H_{38}O_5$
Molecular Weight : 418.5662 g/mole
IUPAC :(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-1-yl 2,2-dimethylbutanoate.
Category : **Anticholestermicagents, hypolipidemic agents.**

Ezetimibe

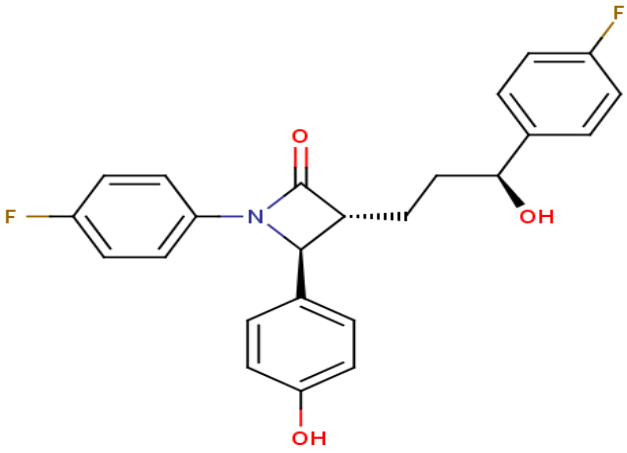

Figure 1. Structure of Ezetimibe

Chemical formula : $C_{24}H_{21}F_2NO_3$
Molecular Weight : 409.4252 g/mole
IUPA: (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one
Category : Cholestrol absorption inhibitors.

METHOD VALIDATION

System Suitability

A Standard solution was prepared by using Simvastatin and Ezetimibe working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Simvastatin and Ezetimibe**,** retention times and peak areas.

Acceptance Criteria

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Simvastatin and Ezetimibe peaks is NLT 3000.
4. The Tailing factor (T) for the Simvastatin and Ezetimibe peaks is NMT 2.0

Observation

The %RSD for retention times and peak areas were found to be within the limit.

Specificity

Simvastatin and Ezetimibe

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Acceptance Criteria

Chromatograms of standard and sample should be identical with near Retention time.

Observation

The chromatograms of Standard and Sample were same identical with same retention time.

Precision

Repeatability

a. System precision: Standard solution prepared as per test method and injected five times.

b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Acceptance Criteria

The % relative standard deviation of individual Simvastatin and Ezetimibe, from the six units should be not more than 2.0%. The individual assays of Simvastatin and Ezetimibe should be not less than 98% and not more than 102.0%.

Observation

Test results are showing that the test method is precise. Intermediate precision (analyst to analyst variability)

A study was conducted by two analysts as per test method

Acceptance Criteria

The individual assays of Simvastatin and Ezetimibe should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Observation

Individual %assays and % RSD of Assay are within limit and passes the intermediate precision,

Accuracy (Recovery)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Simvastatin and Ezetimibe into each volumetric flask for each spike level to get the concentration of Simvastatin and Ezetimibe equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Simvastatin and Ezetimibe were calculated.

Acceptance Criteria

The mean % recovery of the Simvastatin and Ezetimibe at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy.

Linearity of Test Method

A Series of solutions are prepared using Simvastatin and Ezetimibe working standards at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times [6-12].

Acceptance Criteria

- Correlation Coefficient should be not less than 0.9990.
- % of y- Intercept should be ±2.0.
- % of RSD for level 1 and Level 6 should be not more than 2.0%.

Observation

The linear fit of the system was illustrated graphically.

Ruggedness of Test Method

System to system variability

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Acceptance Criteria

The % relative standard deviation of Simvastatin and Ezetimibe from the six sample preparations should be not more than 2.0% the % assay of Simvastatin and Ezetimibe should be between 98.0%-102.0%.

Observation

The % RSD was found within the limit. Ref tables: 3 &7.

Column to column variability

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analyzed as per test method

Acceptance Criteria

The %RSD of Simvastatin and Ezetimibe tablets should be NMT2.0%. The %assay of Simvastatin and Ezetimibe should be between 98.0% and 102.0% for individual drugs.

Observation

The results obtained by comparing with both two types were within limit. Refer tables: 3 &9

Robustness

Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Simvastatin and Ezetimibe and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

Acceptance Criteria

The Tailing Factor of Simvastatin and Ezetimibe standards should be NMT 2.0 for Variation in Flow.

Observation

The tailing factor for Simvastatin and Ezetimibe was found to be within the limits.

Effect of variation of temperature

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc.

Similarly sample solution was chromatographed at 25ºC temperature. Simvastatin and Ezetimibe were resolved from all other peaks and the retention times were comparable with those

Acceptance Criteria

The Tailing Factor of Simvastatin and Ezetimibe standard and sample solutions should be NMT 2.0 for Variation in temperature [12-13].

Limit of Detection and Quantitation (LOD and LOQ)

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

RESULTS AND DISCUSSION

Method development

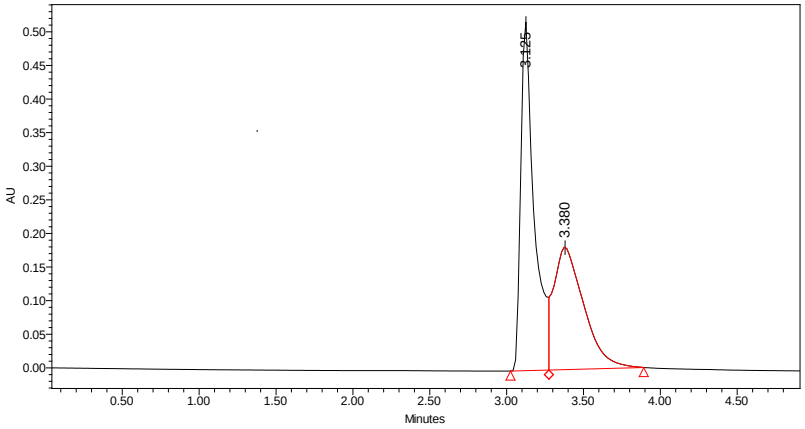

Fig 3: Chromatogram of Trial 1

Table 2: Retention peak of Simvastatin and Ezetimibe

S.No	Name of the peak	Retention time(min)
1	**Simvastatin**	3.125
2	**Ezetimibe**	3.380

Optimized Method

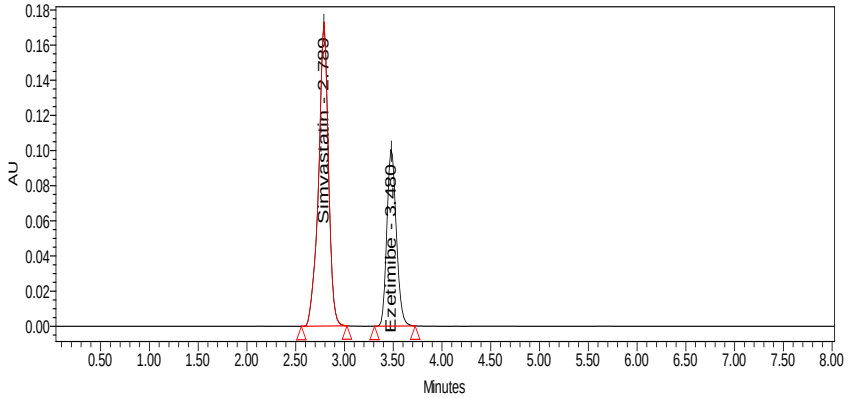

Fig 4: Chromatogram of standard

Table 3: Retention peak of Simvastatin and Ezetimibe

S.No	Name of the peak	Retention time(min)
1	**Simvastatin**	2.789
2	**Ezetimibe**	3.480

System Suitability

Table 5: Data of System Suitability for Simvastatin

Injection	RT	Peak Area	USP Plate count	USP Tailing
Mean	2.789	1228070.6	8891.11744	0.889742
SD	0.00707	21061.68	-------	-------
% RSD	0.025353	1.71	-------	-------

Table 4: Data of System Suitability for Ezetimibe

Injection	RT	Peak Area	USP Plate count	USP Tailing
Mean	3.479	677206.8	6109.379	1.154635
SD	0.001817	7252.993	-------	-------
% RSD	0.05221	1.071016	-------	-------

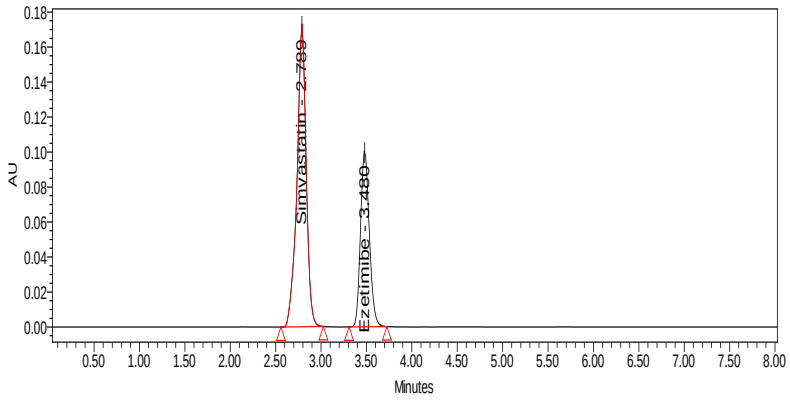

Fig 5: Chromatograms of system suitability

Specificity

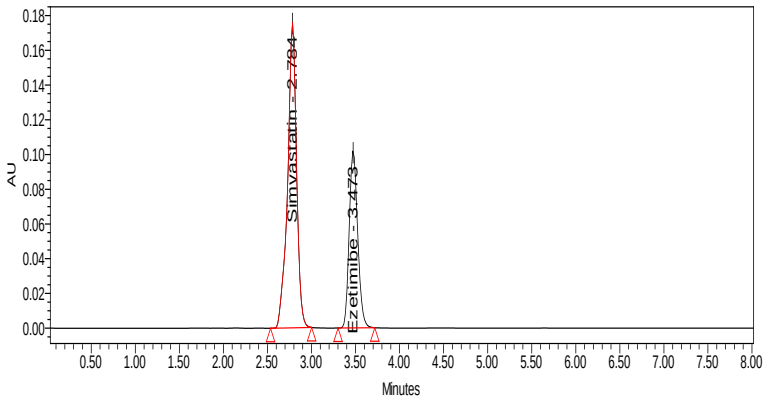

Fig 6: Chromatogram of standard

Ezetimibe

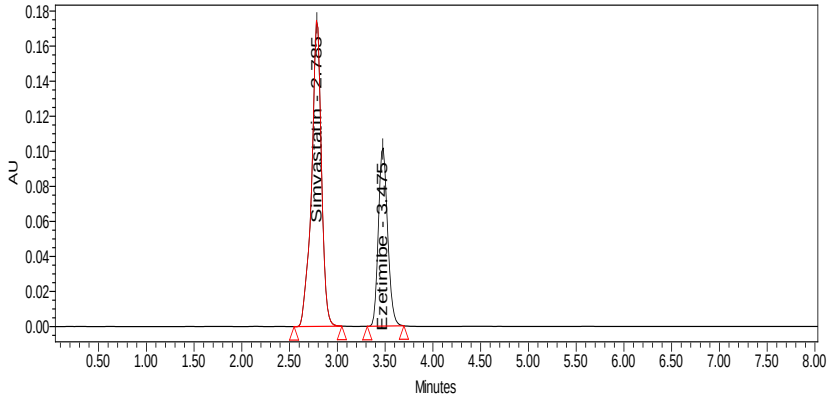

Fig 7: Chromatogram of sample

Precision

Repeatability- System precision

Table 6: Data of Repeatability (System precision) for Simvastatin

	Injection	Peak Areas of Simvastatin	%Assay
Concentration 40ppm	1	1239704	99.95
	2	1246846	100.24
	3	1252530	100.06
	4	1261073	99.30
	5	1266667	100.00
Statistical Analysis	**Mean**	1253364	99.91
	SD	10795.53	0.35819
	% RSD	0.861324	0.35

Table 7: Data of Repeatability (System precision) for Ezetimibe

	Injection	Peak Areas of Ezetimibe	%Assay
Concentration 40ppm	1	676488	98.66
	2	683935	99.30
	3	686924	101.53
	4	687698	100.53
	5	694665	99.98
Statistical Analysis	**Mean**	685942	100.00
	SD	6586.819	1.107678
	% RSD	0.960259	1.10

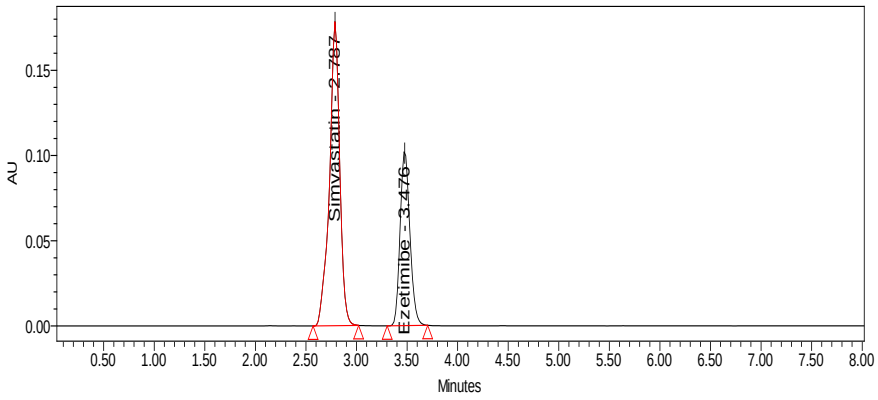

Fig 8: Chromatograms of system

Method precision

Table 8: Data of Repeatability (Method precision) for Simvastatin

	Injection	Peak Areas of Simvastatin	%Assay
Concentration 40ppm	1	1214943	98.6
	2	1220150	99.02
	3	1220212	98.12
	4	1219505	98.31
	5	1265543	98.81
	6	1220150	98.36
Statistical Analysis	**Mean**	1226751	98.48
	SD	19113.65	0.352647
	% RSD	1.558071	0.35

Table 9: Data of Repeatability (Method precision) for Ezetimibe

	Injection	Peak Areas of Ezetimibe	%Assay
Concentration 40ppm	1	674665	98.55
	2	672015	98.88
	3	672211	99.40
	4	677612	99.30
	5	689531	100.53
	6	672015	98.28
Statistical Analysis	**Mean**	676341.5	99.278
	SD	6824.749	0.827236
	% RSD	1.009068	0.83

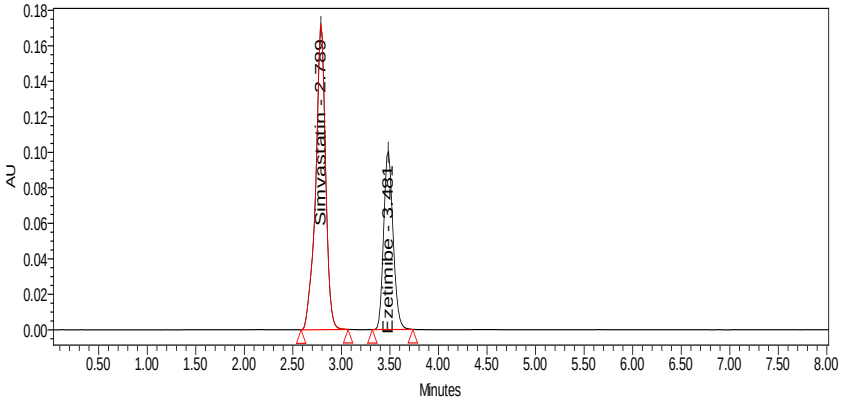

Fig 9: Chromatograms of Repeatability

Accuracy (Recovery)

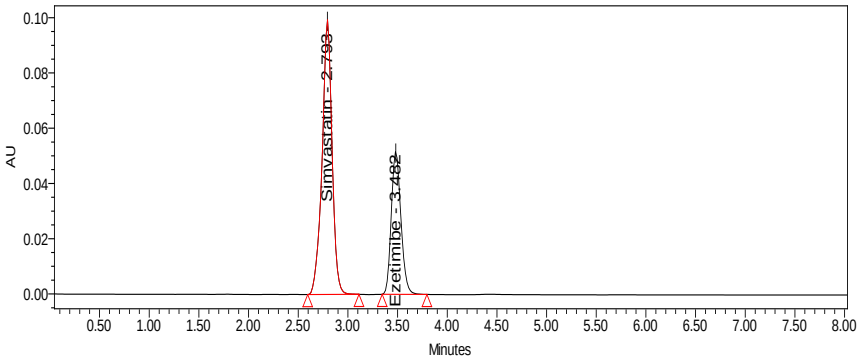

Fig 10: Chromatograms for accuracy (50%)

Linearity

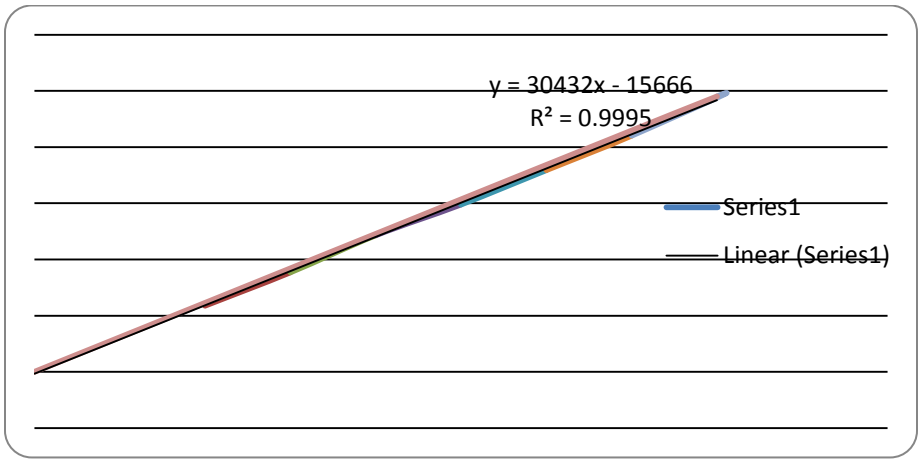

Fig 11: Linearity Plot (Concentration Vs Response) of **Simvastatin**

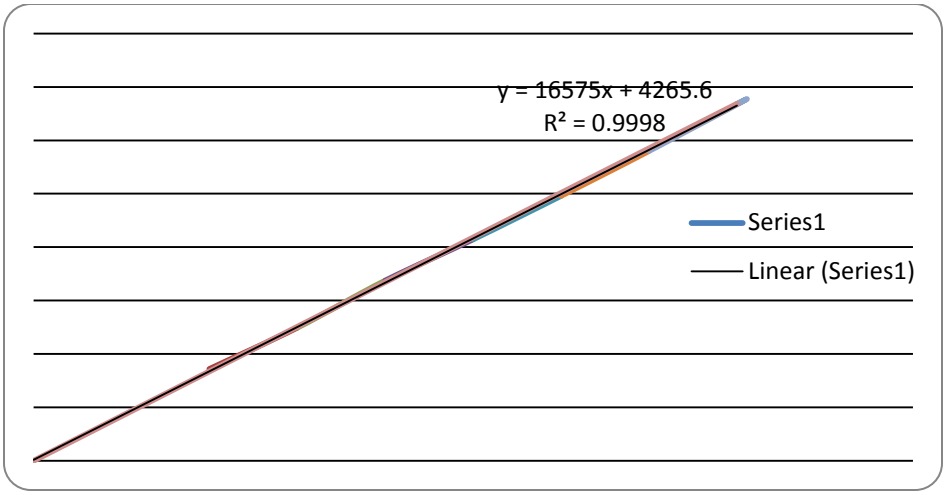

Fig 12: Linearity Plot (Concentration Vs Response) **of Ezetimibe**

Ruggedness- System to System variability

Table 10: Data of system to system variability (Simvastatin)-System-2

S.NO:	Peak area	Assay % of Simvastatin
Mean	1230010	99.07667
%RSD	1.579467	0.56

Table 11: Data of system to system variability (Ezetimibe)- System-2

S.NO:	Peak area	Assay % of Ezetimibe
Mean	677087	98.64
%RSD	0.959095	0.12

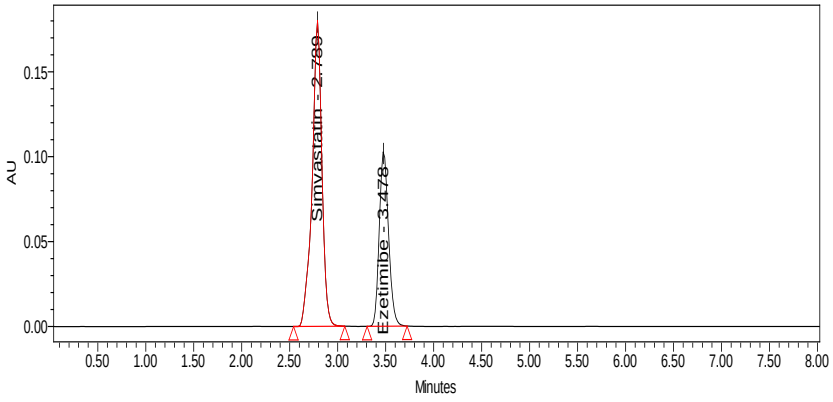

Fig 13: Chromatogram of system to system variability

Robustness

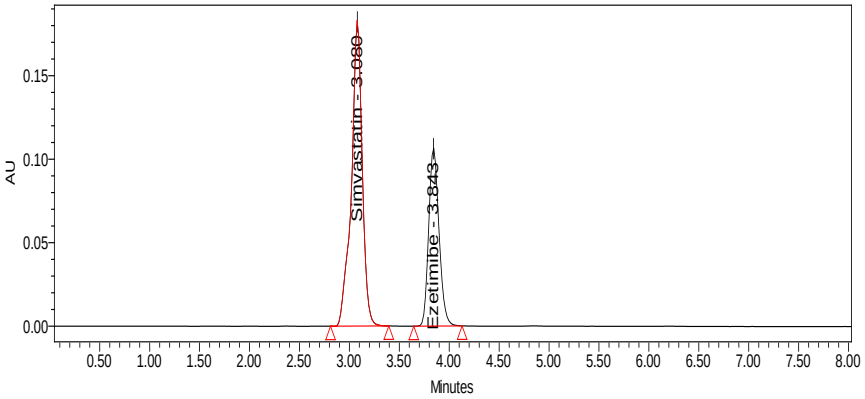

Fig 14: Chromatograms of robustnesso

Limit of Detection and Limit of Quantitation (LOD and LOQ)

Simvastatin

From the linearity plot the LOD and LOQ are calculated

LOD = 0.22

LOQ = 0.69

Ezetimibe

LOD = 0.25

LOQ = 0.75

CONCLUSION

The developed methods were validated as per ICH guidelines and were found to be within the prescribed limit. It concludes that the developed methods are simple, accurate, sensitive and precise and suitable for both authentic and tablet dosage form.

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