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Research Article

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF EZETIMIBE & SIMVASTATIN IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

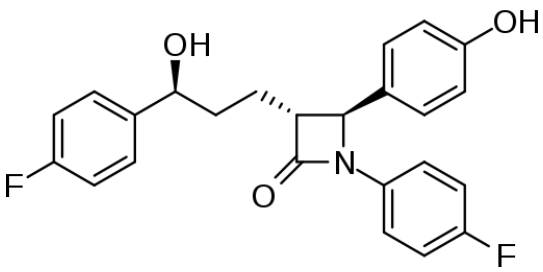
A simple, sensitive, specific and economic chromatographic method was developed through a Sunfire C_{18} (250) column , Mobile phase used was Acetonitrile: Phosphate buffer (60:40%), at the flow rate of 1.8ml/min, Ezetimibe & Simvastatin were eluted at acceptable retention times of 2.34 and 7.35 minutes respectively with good resolution by monitoring UVdetection at 225nm .Throughout the separation the drugs were stable, the studies were carried out by attempting deliberate degradation of the sample with exposure to stress conditions like acidic (1M HCl), alkaline (1M NaOH), 105^{0}C Heat, Oxidizing agents (H_2O_2) and Water. This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for specificity, selectivity, accuracy and stability. This method was fast, reliable and stable for the accurate determination of Ezetimibe & Simvastatin in formulation by RP-HPLC[1,2].

KEY WORDS

Ezetimibe,Simvastatin ,RP-HPLC

INTRODUCTION[3]

Ezetimibe,chemically(3R,4S)1(4fluorophenyl)-[(3S)-3-(4-fluorophenyl)-3hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one, molecular formula is $C_{24}H_{21}F_2NO_3$, Molecular weight 409.4 and it is highly soluble in alcohols (methanol, ethanol, 1-propanol), it acts as Anticholesteremic[4] and Cholesterol Absorption Inhibitor.



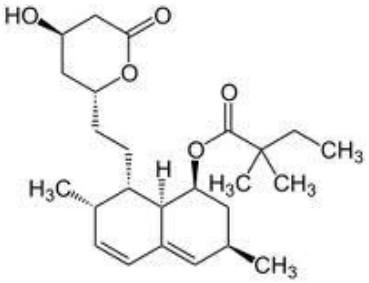


Figure No.1&2. Structure of Ezetimibe& Simvastatin

Simvastatin: Chemically(1S,3R,7S,8S,8aR)-8-(2-((2R,4R)-4-hydroxy-6-oxooxan-2yl)ethyl)-3,7dimethyl1,2,3,7,8,8ahexahydronapthalen1 yl2,2dimethylbutanoat, Molecular formula $C_{25}H_{38}O_5$, Molecular weight 418.5662, it is Soluble in water, acts as Anticholesteremic and Antilipemic. Various analytical methods have been reported for the assay of ezetimibe and simvastatin individually or com bination with other drugs in biological samples/formulations.

They include HPLC[5,6], highperformance thin la yer chromatography, derivative UV spectroph otometry. Literature survey[7-15] reveals that no analytical method for determination for this combined dosage forms is reported with stable components and with very good resolution. So it is felt worthwhile to develop a simple, rapid, accurate, precise and more economical high performance liquid chromatographic method for simultaneous estimation of ezetimibe and simvastatin in bulk and its combined dosage form.

MATERIALS AND METHODS

HPLC instrument: HPLC system (WATERS)
Series: Alliance e2695
Soft Ware: Empower
Column: SunFire C_{18} ((250mm, 4.6mm, 5µ)
Vacuum filter: Model XI 5522050 of Millipore
Potassium dihydrogen orthophosphate :(Merck – HPLC grade)
Orthophosphoricacid :(Merck – HPLC grade)
Ammonium :(Merck-GR)
Methanol: (Merck HPLC grade)
Acetonitrile :(Merck – HPLC grade)
Water: MilliQ water

Preparation of 0.05M of Potassium dihydrogen Phosphate Buffer Solution (pH 7.2):

6.8 g of Potassium dihydrogen Phosphate was dissolved in 1000ml of Milli Q water. The solution was adjusted to a PH of 7.2 with Triethylamine. Then it was degassed in ultrasonicator for 10 minutes and then filtered through 0.45 µ pore size membrane filter.

Preparation of mobile phase[16, 17]:

Mix a mixture of above buffer 400 ml and 600 ml of Acetonitrile HPLC grade and degas in ultrasonic water bath for 10 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of standard solution of Ezetimibe and Simvastatin:

10mg of Simvastatin and 10mg of Ezetimibe working standard were taken in 100ml volumetric flask. It was dissolved in 50ml methanol and made up to the mark with the methanol to get a concentration of 100µg/ml and 100µg/ml. It was degassed in ultrasonicator and then filtered through membrane filter of 0.45µ pore size.

Preparation of sample solution of Ezetimibe and Simvastatin:

10 tablets were crushed and powder equivalent to 10mg was taken into 100ml volumetric flask .It was made to dissolve with methanol 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.

RESULTS AND DISCUSSION:

METHOD OPTIMIZATION[18]

Optimization of the method done by performing various trials by change in mobile phase composition, column[19], flowrate, etc.

OPTIMISED CHROMATOGRAPHIC CONDITIONS	
Mode of separation	Isocratic elution
Mobile phase	ACN: Phosphate buffer (60%: 40%)
Column	Sunfire C_{18} (250mm,4.6mm(id), 5µ)
Flow rate	1.8 ml/min
Detector wavelength	225 nm
Injection volume	15µl
Oven temperature	Ambient
Run time	9min

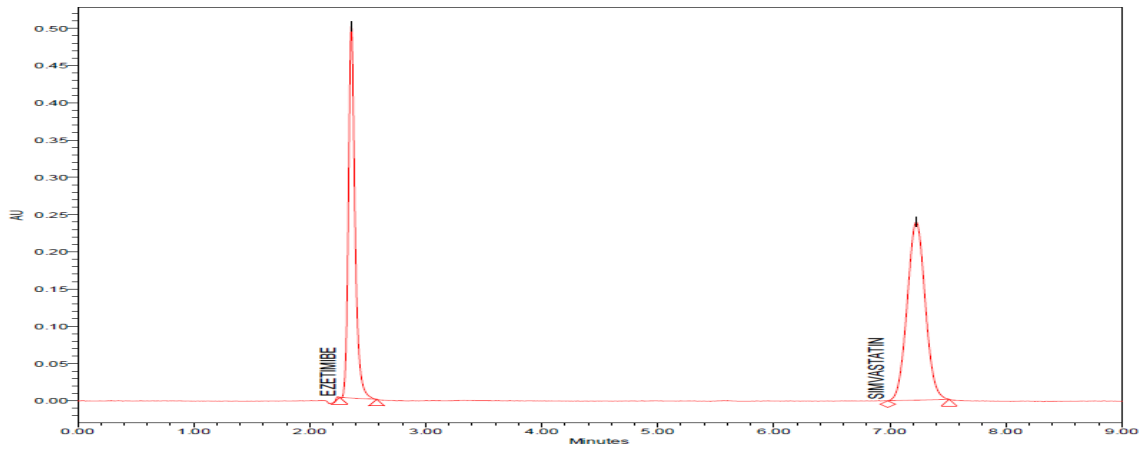


Figure No.3: Chromatogram for optimized conditions (Ezetimibe & Simvastatin)

METHOD VALIDATION

SYSTEM SUITABILITY[20]

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the system suitability tests: capacity factor, tailing factor and theoretical plates.system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram

obtained from reference substance solution is presented. System suitability parameters were shown in **Table.1**. Standard chromatogram was given in **Figure no 1**.

Parameters	Ezetimibe	Simvastatin
Tailing factor (T)	1.3	1.0
Number of theoretical plate(n)	8683	10051
Retention time (R	2.35	7.23
%RSD	0.7	0.4

Table No.1: System suitability parameters

SOLUTION STATE STABILITY

Stability of samples is determined at different time intervals like at zero, 12th, 24th hours. The samples were stable up to 24 hours. The results were shown in **Table No 2** at 24th hour:

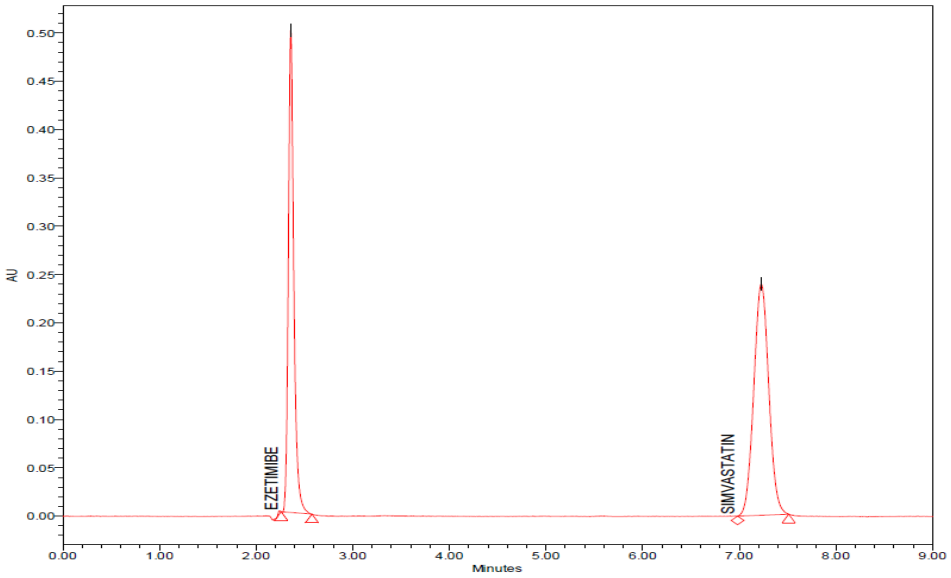


Figure no. 4: chromatogram of sample Ezetimibe and Simvastatin

	Compound	RT	Area	% Assay
0 hour	Ezetimibe	2.34	2087273	100.12
0 hour	Simvastatin	7.35	2696175	100.45
12th hour	Ezetimibe	2.38	2062054	99.92
12th hour	Simvastatin	7.43	2687436	100.12
24th hour	Ezetimibe	2.35	2047654	99.22
24th hour	Simvastatin	7.36	2679056	99.96

Table No.2: Solution State Stability

SPECIFICITY

The Specificity[21] for these two drugs was determined by using 0.1N HCl, 0.1N NaOH and 1%H_2O_2 and upon refluxing drug solution at 60^0c for 30min when drug was mixed with 0.1N HCL, 0.1N NaOH and 1% H_2O_2 upon refluxing to 60^0c. It was found to be occurrence of irregular peak and peak elution was not good as shown in **Table No.3** and results has shown good Specificity.

S.No	Sample Weight (mg)	Ezetimibe Area	Simvastatin Area	% Assay of Ezetimibe	% Assay of Simvastatin
Acid-Degradation	173.00	2000235	2121378	96.92	96.56
Base-Degradation	173.00	2001354	2212143	95.54	95..52
Peroxide-Degradation	173.00	2001187	2122465	94.54	92.26
Water-Degradation	173.00	2001298	2131764	96.89	96.36
Heat-Degradation	173.00	2001265	2123476	96.24	96.78

Table No.3: The Specificity for two drugs for different Degradation

PRECISION

The Precision[22,23] has done in two ways i.e., System Precision, Method Precision, Intra-day Precision and Inter-day Precision. The %RSD values of Ezetimibe & Simvastatin for System Precision, Method Precision, Intra-day Precision and Inter-day Precision was found in **Table 4 and 5**. Which were in the acceptance limit of less than 2%.

Average Assay	99.96	98.99
%RSD	0.56	0.79

Table No.4: System Precision

METHOD PRECISION

Average Assay	99	98
%RSD	0.65	0.83

Table No. 5: Method Precision

ACCURACY

Accuracy was confirmed by Recovery Studies. The % recovery of Ezetimibe & Simvastatin was found to be 99 %, 100 % which were in the acceptance limit of 98 to 102% as shown in **Table No.6-10.**

Inj.Sample	Spike level	Sample Weight (mg)	Area	Amount added	Amount recovered	% recovered	Mean recovery
Ezetimibe	50% -1	86.50	1043899	49.500	50.08	101	101%
	50% -2	86.50	1055178	49.500	50.62	102	
	50% -3	86.50	1055188	49.500	50.62	102	
	50% -4	86.50	1037779	49.500	49.79	101	
	50% -5	86.50	1046267	49.500	50.19	101	
	50% -6	86.50	1032724	49.500	49.54	100	

Table No.6: Recovery Studies At 50% level for Ezetimibe

Inj.Sample	Spike level	Sample Weight (mg)	Area	Amount added	Amount recovered	% recovered	Mean recovery
Simvastatin	50% -1	86.50	1332759	49.00	49.12	100	100%
	50% -2	86.50	1324327	49.00	48.81	100	
	50% -3	86.50	1343176	49.00	49.50	101	
	50% -4	86.50	1333817	49.00	49.16	100	
	50% -5	86.50	1322444	49.00	48.74	99	
	50% -6	86.50	1339537	49.00	49.37	101	

Table No.7: Recovery Studies At 50% level for Simvastatin

Inj.Sample	Spike level	Sample Weight (mg)	Area	Amount added	Amount recovered	% recovered	Mean recovery
Ezetimibe	100%-1	173	2058276	99	98.74	100	99%
	100%-2	173	2043824	99	98.05	99	
	100%-3	173	2037978	99	97.77	99	
Simvastatin	100%-1	173	2656926	98	97.92	100	100%
	100%-2	173	2643234	98	97.41	99	
	100%-3	173	2678887	98	98.73	101	

Table No.8: Recovery Studies At 100% level

Inj.Sample	Spike level	Sample Weight (mg)	Area	Amount added	Amount recovered	% recovered	Mean recovery
Ezetimibe	150% -1	259.50	3085928	148.500	148.04	100	99%
	150% -2	259.50	3070953	148.500	147.32	99	
	150% -3	259.50	3061148	148.500	146.85	99	
	150% -4	259.50	3056523	148.500	146.63	99	
	150% -5	259.50	3018930	148.500	146.83	98	
	150% -6	259.50	3023669	148.500	145.05	98	

Table No. 9: Recovery Studies at 150% level for Ezetimibe

Inj.Sample	Spike level	Sample Weight (mg)	Area	Amount added	Amount recovered	% recovered	Mean recovery
Simvastatin	150% -1	259.50	4038807	147.00	148.85	101	101%
	150% -2	259.50	4025058	147.00	148.34	101	
	150% -3	259.50	4055025	147.00	149.44	102	
	150% -4	259.50	4048088	147.00	149.14	101	
	150% -5	259.50	4053372	147.00	149.38	102	
	150% -6	259.50	4049735	147.00	149.25	102	

Table No.10: Recovery Studies at 150% level for Simvastatin

LINEARITY

The Linearity of Ezetimibe & Simvastatin was carried out at different concentrations ranging from 50-150 µg/ml and correlation coefficient was found to be 1, which indicates that the concentration had given good linearity as shown in **Figure No.3 & 4**.

Linearity curve of Ezetimibe

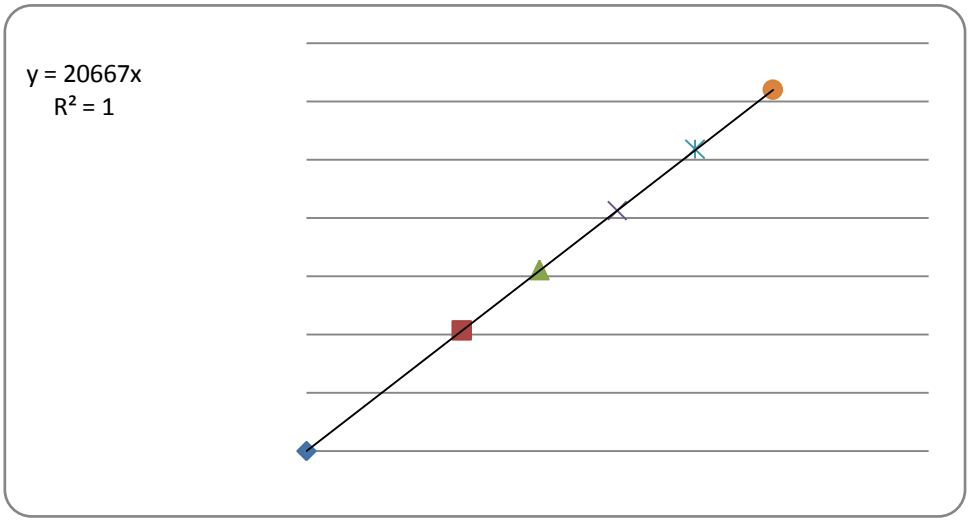


Figure No.5: Linearity curve of Ezetimibe

Linearity of Simvastatin

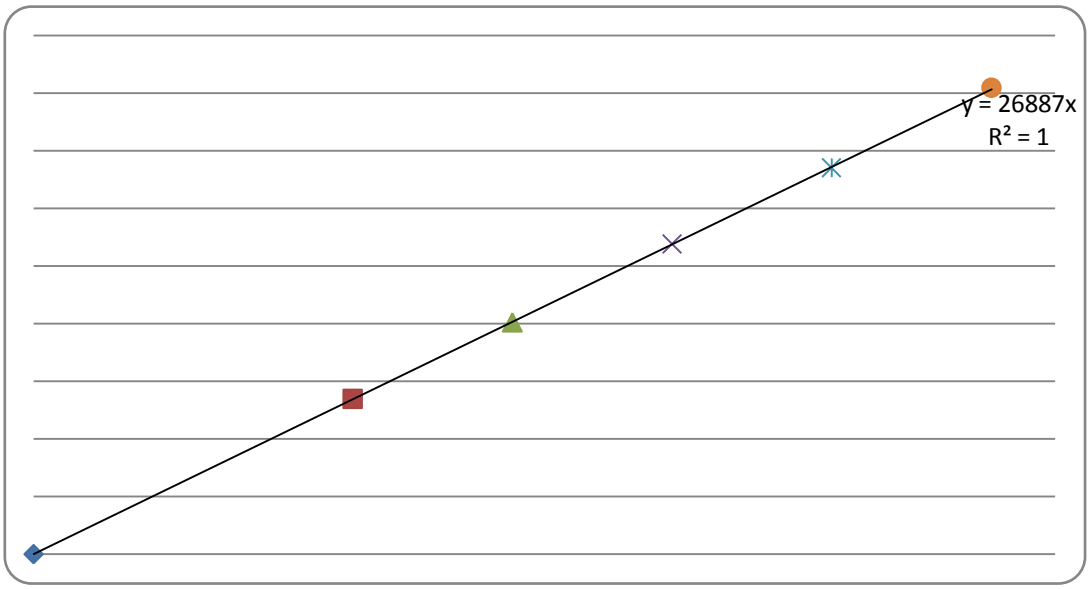


Figure No.6: Linearity curve of Simvastatin

Compound	Standard area	Sample area	Standard weight	Sample weight	Average weight	Label claim	Standard purity
Ezetimibe	2063655	2056299	10.00mg	173.00mg	173.00mg	10mg	99%
Simvastatin	2686295	2668773	10.00mg	173.00mg	173.00mg	10mg	99%

Table No.11: Assay Of Ezetimibe And Simvastatin:

LOD & LOQ of Ezetimibe and Simvastztin

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. The results for LOD&LOQ shown in **Table No.12**

Parameter	LOD	LOQ
Ezetimibe	0.29µg/ml	0.97µg/ml
Simvastatin	0.61µg/ml	2.05µg/ml

Table No.12: LOD & LOQ of Ezetimibe and Simvastztin

ROBUSTNESS

The Robustness of the method developed was validated by changing the flow Rate and Temperature has shown in **Table No: 13**. The selected flow rate and Temperature gives good separation of drugs.

Inj.Sample	Flow Rate (ml/min)	USP Plate Count	USP Tailing	Temperature (^{0}C)	USP Plate Count	USP Tailing
Ezetimibe	1.6	1.30	8883	45	1.36	8414
	2.0	1.33	8619	55	1.32	8565
Simvastatin	1.6	1.0	10851	45	1.34	9098
	2.0	1.03	10138	55	1.30	9733

Table No.13: Robustness for the changes in flow rate and Temperature.

RUGGEDNESS

The proposed method was analyzed by two different analysts by conducting Ruggedness has shown in **Table No.14**. Hence the proposed method has good repeatability.

Compound	Rt	Tailing factor	Number Theoretical Plates
Ezetimibe	2.35	1.3	8683
Simvastatin	7.23	1.0	10051

Table No.14: Robustness for two different analysts.

CONCLUSION

The proposed method was found to be simple, stable, fast, robust, more precise and accurate under the present experimental conditions. Therefore the developed method can be used for routine analysis for simultaneous estimation and stability indicating studies of Ezetimibe and Simvastatin in bulk and pharmaceutical dosage form.

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