International Journal of Farmacia

Journal Home page: www.ijfjournal.com

Formulation of sustained release dosage form of simvastatin and ezetimibe and analytical development by reversed phase high performance liquid chromatography (RP-HPLC)

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Abstract

The present investigation relates to a process of preparing sustained release dosage forms containing simvastatin and ezetimibe in combination and assay by RP-HPLC method development and validation. Chromatographic separation was achieved on a Water's Xterra column (250 x 4.6, 5 microns) using a mobile phase consisting of Potassium di hydrogen phosphate and methanol in the ratio of 70:30 at a flow rate of 0.8 ml per minute. The detection was made at 236 nm. The retention time of ezetimibe and simvastatin were 2.6 and 3.4 minutes respectively. The proposed method was validated for Accuracy, Specificity, Precision, linearity, Robustness, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were studied as per the ICH guidelines.

Key words: Ezetimibe, Simvastatin, Sustained release dosage forms, Reversed phase high performance liquid chromatography, Limit of detection and limit of quantitation.

INTRODUCTION

Simvastatin is chemically (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-dimethylbutanoate. Simvastatin is in a group of drugs called HMG CoA reductase inhibitors, or "statins." It reduces levels of "bad" cholesterol (low-density lipoprotein, or LDL) and triglycerides in the blood, while increasing levels of "good" cholesterol (high-density lipoprotein, or HDL). Ezetimibeis chemically (3*R*,4*S*)-1-(4-fluorophenyl)-3-(4- fluorophenyl)-3- hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. Ezetimibe is a drug that lowers plasma cholesterol levels. It acts by decreasing cholesterol absorption in the small intestine. It may be used alone when other cholesterol-lowering medications are not tolerated, or together with statins (e.g., ezetimibe/simvastatin,) when statins alone do not control cholesterol. Ezetimibe reduces the amount of cholesterol absorbed by the body.The present investigation describes a process of preparing sustained release dosage form containing simvastatin and ezetimibe in combination and assay by RP-HPLC method and its validation.

MATERIALS AND METHODOLOGY

Simvastatin, Ezetimibe, hydrochloric acid, Potassium di hydrogen phosphate, Methanol, Acetonitrile, HPLC grade water, Citric acid, Croscarmellose Sodium(CCS), Hydroxy Propyl Cellulose (HPC), Lactose, Magnesium stearate micro crystalline cellulose (MCC).

Construction of calibration curve of ezetimibe and simvastatin

A calibration curve was plotted over a concentration range of 3-18 µg/mL Simvastatin, 5-30 µg/mL Ezetimibe. The absorbance of each solution was measured at the wavelengths 238.2nm, 243.3nm and

247.6.nm.Calibration curves were constructed for Simvastatin and Ezetimibe by plotting absorbance versus concentrations at both wavelengths.

Validation of assay method

specificity

Specificity is the ability to assess unequivocally the analyze in the presence of components which may be expected to be present. Specificity will be performed to ensure that no co-elution of process impurities and its degradation product exists. Specificity will be demonstrated by injecting the standard solution as per described procedure.

Precision

To experiment the repeatability of standard solution obtained by this method. The precision shall be demonstrated by preparing six standard solutions as per the test method. The precision of method will be evaluated by computing the %RSD of the Area count.

Linearity

Demonstrate the linearity of analyte over the range of 50% to 150% of standard concentration.

Acceptance criteria The correlation coefficient is NLT 0.99.

Accuracy

Accuracy of the test method shall be demonstrated by preparing accuracy samples in 50 mL volumetric flask at the level of 50%, 100%, and 150% of samples concentration. The accuracy samples will be prepared in triplicate in each level.

Acceptance criteria

The Recovery for all levels should be between 98%-102%.

Limit of detection (LOD)

Limit of Detection (LOD) of Ezetimibe and Simvastatin by the proposed methods were determined using calibration standards. LOD were calculated as 3.3 s/S where S is the slope of the calibration curve and s is the standard deviation of y-intercept of regression equation. The low values indicated the good sensitivity of the method proposed.

Limit of quantitation (LOQ)

Limit of Quantitation (LOQ) of Ezetimibe and Simvastatin by the proposed methods were determined using calibration standards.LOQ were calculated as 10 s/S where S is the slope of the calibration curve and s is the standard deviation of y-intercept of regression equation. The low values indicated the good sensitivity of the method proposed.

Robustness

To establish the robustness of test method and to demonstrate its reliability for minor changes in chromatographic conditions Robustness of test method will be demonstrated by carrying out system suitability under normal condition and each of the altered conditions.

Acceptance criteria

Following system suitability parameters to be evaluated should comply with all altered conditions.

Table 1: System suitability parameters for all altered conditions (Robustness)

S. No	Test	Specifications
1.	Tailing of Ezetimibe and Simvastatin in SST solution	NMT 2
2.	Resolution of Ezetimibe and Simvastatin in SST solution	NLT 2
3.	Number of theoretical plates for Ezetimibe and Simvastatin peak in SST solution	NLT 2500

RESULTS AND DISCUSSION

Assay of ezetimibe and simvastatin tablet dosage form

Table 2: System suitability Parameters

Test	Specifications
Resolution R between Ezetimibe and Simvastatin peak	NLT 2
%RSD for area response of Ezetimibe and Simvastatin peak obtained with the six replicate measurement of standard solution	NMT 2.0%
No. of theoretical plates for Ezetimibe and Simvastatin peak in SST	NLT 2500

Similarity factor of Ezetimibe and Simvastatin in SST	0.98-1.02

Percentage recovery

Table 3: Percentage recovery of Ezetimibe in tablet dosage form

Formulation	Label claim (mg/tablet)	10 tablets average weight	Amount obtained by proposed method	Percentage recovery
Ezetimibe	10 mg	1668.86 mg	39.57 µg/ml	99.1%

Table 4: Percentage recovery of Simvastatin in tablet dosage form

Formulation	Label claim (mg/tablet)	10 tablets average weight	Amount obtained by proposed method	Percentage recovery
Simvastatin	10 mg	1668.86 mg	39.83 µg/ml	99.7%

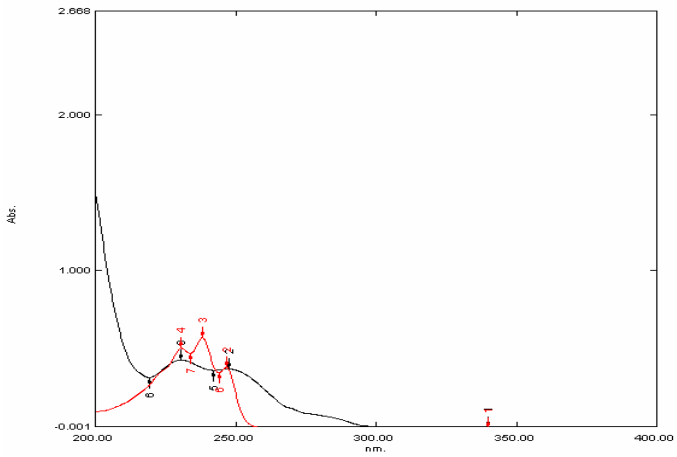

Fig.1: Overlay Spectrum ofSimvastatin andEzetimibe

Standard calibration curve of ezetimibe

It was found that the estimation of Ezetimibe by UV spectrometric method at λ max 247.6 nm in Methanol and Water in the proportion of 40:60.

Standard calibration curve of simvastatin

It was found that the estimation of Ezetimibe by UV spectrometric method at λ max 238.2 nm in Methanol and Water in the proportion of 40:60.

Optimized method

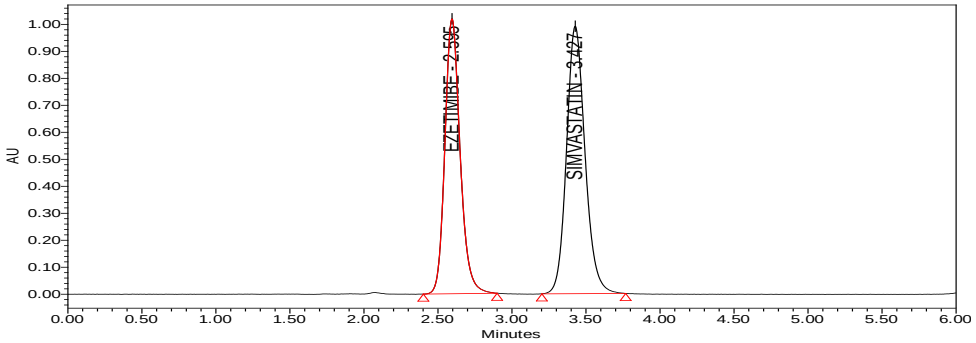

Table 5: Typical Chromatogram of Simvastatin andEzetimibe

S.NO	Name	Retention Time	USP Resolution	USP Tailing	USP Plate Count
1	EZETIMIBE	2.595		1.17	3198
2	SIMVASTATIN	3.427	3.98	1.14	3691

Table 6: Linearity table for Ezetimibe, Simvastatin

Conc. %	ug/ml	Area -Ezetimibe	Area- Simvastatin	% Assay - Ezetimibe	% Assay - Simvastatin
50	40	3641909	4328072	99	100
75	60.00	5474028	6482081	99	100
100	80.00	7297696	8658889	99	100
125	100	9120594	10802288	99	100
125	100	9120594	12986031	99	100

Response is linear over the concentration range from 50% to 150% of test concentration

Table 7: Accuracy table for - Ezetimibe

Ezetimibe Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	250.33	3643247	39.640	39.57	100	100
50%	250.33	3645687	39.640	39.59	100	
50%	250.33	3641923	39.640	39.55	100	
50%	250.33	3644581	39.640	39.58	100	
50%	250.33	3640699	39.640	39.54	100	
50%	250.33	3646838	39.640	39.61	100	
100%	500.66	7291179	79.280	79.19	100	100
100%	500.66	7295231	79.280	79.23	100	
100%	500.66	7289402	79.280	79.17	100	
150%	750.99	10914097	118.920	118.53	100	100
150%	750.99	10912145	118.920	118.51	100	
150%	750.99	10984258	118.920	119.29	100	
150%	750.99	10994580	118.920	119.41	100	
150%	750.99	10918676	118.920	118.58	100	
150%	750.99	10915544	118.920	118.55	100	

Table 8: Accuracy table for – Simvastatin

	Simvastatin					
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	250.33	4320798	39.880	39.83	100	
50%	250.33	4321379	39.880	39.83	100	
50%	250.33	4328802	39.880	39.90	100	100
50%	250.33	4321578	39.880	39.84	100	
50%	250.33	4327013	39.880	39.89	100	
50%	250.33	4326989	39.880	39.89	100	

100%	500.66	8652379	79.760	79.76	100	
100%	500.66	8642852	79.760	79.67	100	100
100%	500.66	8651434	79.760	79.75	100	
150%	750.99	12976891	119.640	119.62	100	
150%	750.99	12911408	119.640	119.02	99	
150%	750.99	12910234	119.640	119.01	99	100
150%	750.99	12987883	119.640	119.72	100	
150%	750.99	12906798	119.640	118.98	99	
150%	750.99	12991328	119.640	119.76	100	

Acceptance criteria

The Recovery for all levels of accuracy should be between 97%- 103%.

The recovery results indicating that the method has an acceptable level of accuracy for the assay of Ezetimibe and Simvastatin from 50% to 150% of test concentration (Fig: 8.35-8.49 & Table: 8.18-8.25).

LOD and LOQ

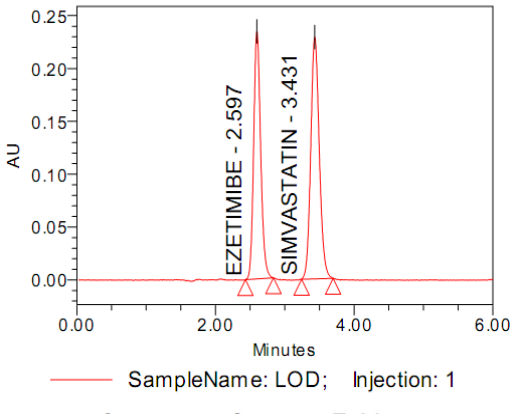

Fig. 2: Typical Chromatogram of LOD

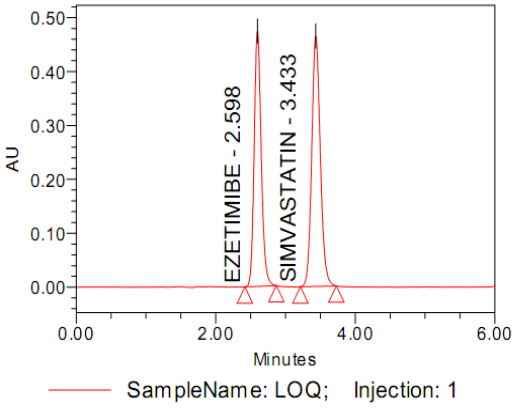

Fig. 3: Typical Chromatogram of LOQ

Table 9: Summary Table of LOD and LOQ - Ezetimibe

Component Summary Table
Name: EZETIMIBE

	SampleName	Inj	Name	RT	Area
1	LOD	1	EZETIMIBE	2.597	1640776
2	LOQ	1	EZETIMIBE	2.598	3337520
Mean					
Std. Dev.					
% RSD					

Table 10: Table for LOD and LOQ - Simvastatin

Analyte	LOD Concentration (µg/ml)	LOD S/N ratio	LOQ Concentration (µg/ml)	LOQ S/N ratio
Simvastatin	40	2.1317	40	7.1058

The low values indicated the good sensitivity of the method proposed

Table 11: Table of Robustness

S.No	Altered Conditions	RT- Ezetimibe	RT - Simvastatin	Area- Ezetimibe	Area - Simvastatin
1	TEMP1	2.595	3.434	7203005	8428624
2	TEMP2	2.585	3.391	7158913	8351520
3	FLOW1	3.231	4.275	9076098	10584420
4	FLOW2	2.165	2.859	5880030	6860347
MEAN				7329511.5	8556227.75
STD				1316139.4	1532727.725
% RSD				0.03%	0.03%

The experiment proves the reliability of the test method for minor changes in the chromatographic conditions.

CONCLUSION

In the present study, a new RP-HPLC method is developed for the estimation of Simvastatin and Ezetimibe which is simple, less time consuming using an economical column. The analysis is resolved by using Potassium di hydrogen phosphate: Methanol (70:30) as mobile phase at a flow rate of 0.8 ml/min using Water's Xterra column (250 x 4.6; 5µ) on an isocratic HPLC system. There was no interference observed from other impurities and Simvastatin and Ezetimibe has been eluted with good peak shape, response within 6 min. The assay of the sample has been carried out using this new method and it was found to be 99.1% and 99.7% respectively. The developed method has been validated for different parameters like Specificity, Precision, Linearity, Accuracy, Robustness, LOD and LOQ. It was concluded that the order of extending the release of the drug increase with the increase in the coating concentration of the polymer. All the results obtained were within the acceptance limits indicating that the developed method is simple, specific accurate and economical.

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