Journal of Pharmaceutical and Biomedical Analysis Letters

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Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Ezetamibe acid and Simvastatin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Ezitamibe and Simvastatin by using Agilent C18 5µm (4.6*250mm) column, the flow rate was 1ml/min, mobile phase ratio was Methanol: ACN (70:30%v/v), detection wave length was 238nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.443 mins and 2.918 mins. The % purity of Ezetamibe and Simvastatin was found to be 100.7% and 101.4% respectively. The system suitability parameters for Ezetamibe and Simvastatin such as theoretical plates and tailing factor were found to be 1.7, 2114.5and 1.7, 2931.0 the resolution was found to be 8.0.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ezetamibe and Simvastatin was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 2.0 and 2.0, % RSD for intermediate precision was 1.5 and 1.1 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ezetamibe and Simvastatin in API and Pharmaceutical dosage form.

Keywords: Ezetamibe, Simvastatin, HPLC

A R T I C L E I N F O

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Article History: Received 29 March 2016, **Accepted** 11 May 2016, **Available Online** 18 July 2016

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Manuscript ID: JPBMAL3048

PAPER-QR CODE

Citation: Y. Krishna Reddy, *et al.* Analytical Method Development and Validation for the Simultaneous Estimation of Ezetamibe acid and Simvastatin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form. *J. Pharm, Biomed. A. Lett*., 2016, 4(2): 86-92.

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1. Introduction

Analytical methods:

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5].

Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate[7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

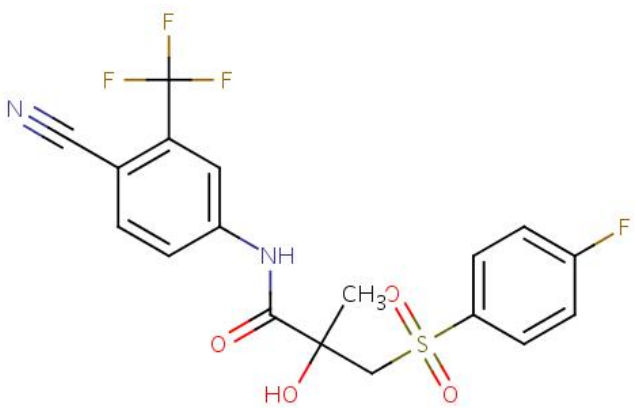


Figure 1: Ezetamibe

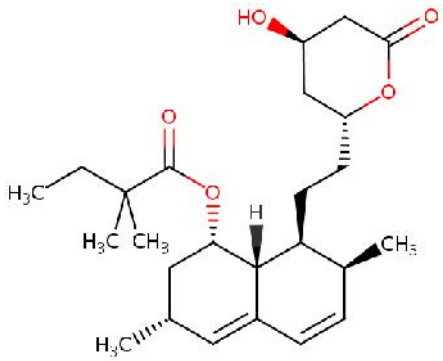


Figure 2: Simvastatin

2. Materials and Methods

Apparatus: The instrument used for the study was Waters, Empower 2695 modual, PDA detector. Empower 2 software.

Reagents and Materials:

The solvents used were Potassium dihydrogen orthophosphate, Methanol, Acetonitril and Water [10].

Selection of detection wavelength:

10 mg of Ezetamibe and Simvastatin was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Ezetamibe and Simvastatin. The isobestic point was taken as detection wavelength.), detection wave length was 238nm.

Selection of mobile phase

Methanol: ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved [11].

Optimization Chromatographic trials for Simultaneous Estimation of Ezetamibe and Simvastatin by RP-HPLC.

Optimization Chromatographic conditions

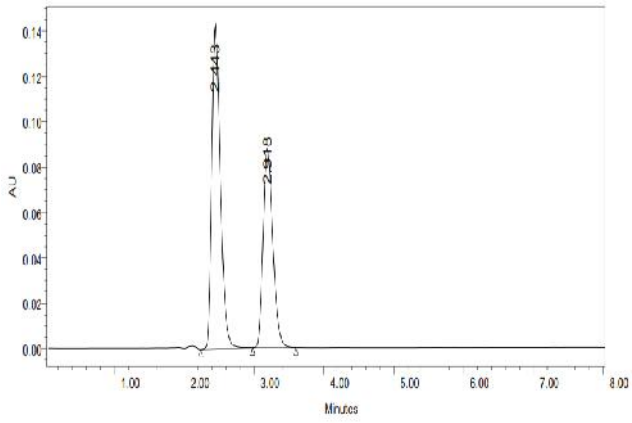


Figure 3: Optimization Chromatogram

Column : Agilent C18 5µm (4.6*250mm)
Mobile phase ratio : Methanol: ACN (70:30%v/v)
Detection wavelength : 238 nm
Flow rate : 1.0ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10min
Retention time : 2.443 and 2.918 mins

Observation:

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Procedure

Preparation of mobile phase

A mixture of Methanol 700ml (70%), 300 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration [12].

Preparation of the individual Ezetamibe standard preparation:

10mg of Ezetamibe working standard was accurately weighed and transferred into a 10mlclean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further10.0mlfrom the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant[13].

Preparation of the individual Simvastatin standard preparation:

10mg of Simvastatin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant[14].

3. Results and Discussion

Method Validation Parameters

1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

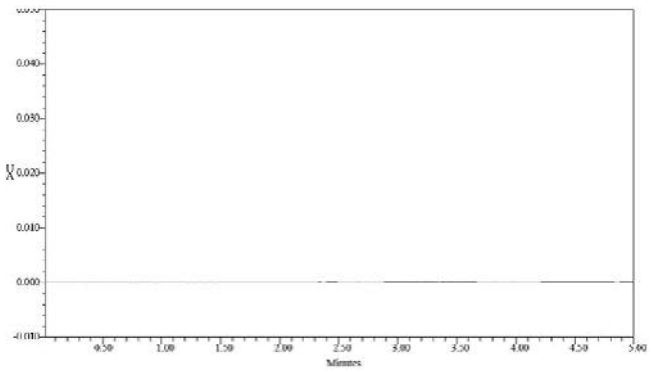


Figure 4: Chromatogram of Blank

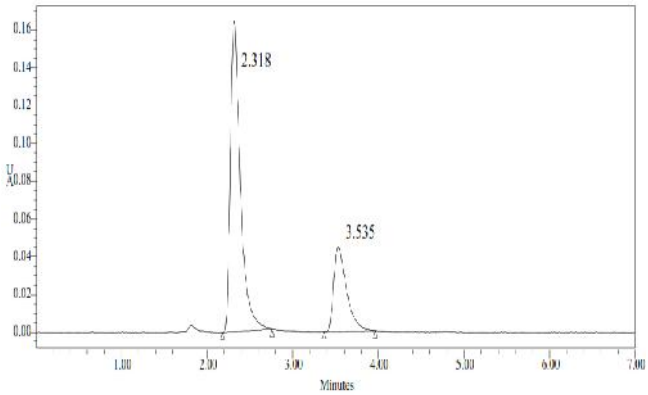


Figure 5: Chromatogram of Sample

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The calibration curve was obtained by plotting the concentration vs. peak area [15].

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively [16].

Acceptance criteria: The mean % recovery of the Ezetimibe and Simvastatin at each level should be not less than 95.0% and not more than 105.0%.

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported [17].

Repeatability:

The precision study was performed for five injections of Ezetamibe and Simvastatin. Each standard injection was

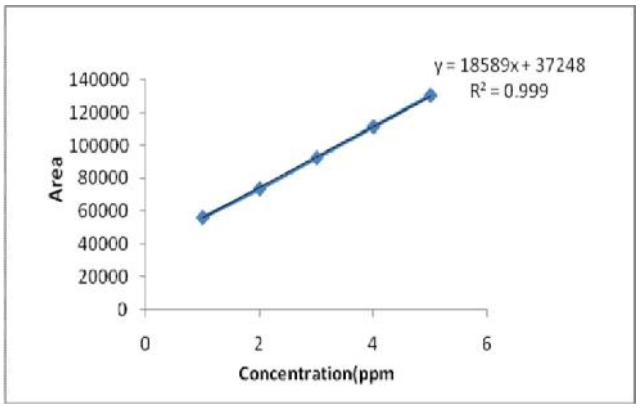
injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD.

Intermediate Precision:

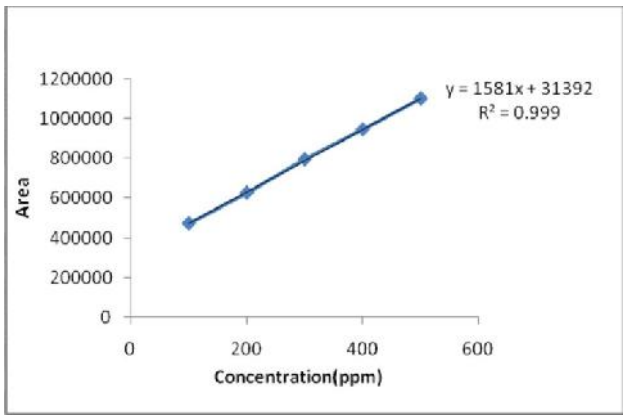
The intermediate precision study was performed for five injections of Ezetamibe and Simvastatin. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD [18].

Validation of the Method

Linearity: The linearity study was performed for the concentration of 1 ppm to 5 for Ezetimibe and 100ppm to 500ppm for Simvastatin and chromatograms are shown below [19].



Calibration graph of Ezitimibe



Calibration graph of Simvastatin

Recovery studies

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated [20].

Detection Limit

The LOD was performed for Ezetimibe and Simvastatin was found to be 2.95and 3.04 respectively [21, 22].

Quantitation Limit

The LOQ was performed for Ezetimibe and Simvastatin was found to be 9.87 and 10 respectively.

4. Conclusion

A new method was established for simultaneous estimation of Ezetamibe and Simvastatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ezetamibe and Simvastatin by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Methanol: ACN (70:30%v/v), detection wave length was 238nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.

The retention times were found to be 2.443 mins and 2.918 mins. The % purity of Ezetamibe and Simvastatin was found to be 100.7% and 101.4% respectively. The system suitability parameters for Ezetamibe and Simvastatin such as theoretical plates and tailing factor were found to be 1.7, 2114.5and 1.7, 2931.0 the resolution was found to be 8.0.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ezetamibe and Simvastatin was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 2.0 and 2.0, % RSD for intermediate precision was 1.5 and 1.1 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ezetamibe and Simvastatin in API and Pharmaceutical dosage form.

Table 1: Calibration data of Ezetimibe and Simvastatin

	PeakName	RT	Area	Height
1	Simvastatin	2.297	869216	109198
2	Simvastatin	2.264	1148093	145069
3	Simvastatin	2.308	1398858	164962
4	Simvastatin	2.370	1676584	193291
5	Simvastatin	2.322	1936686	238262
6	Bicalutamide	3.458	296156	30269
7	Bicalutamide	3.351	371946	39434
8	Bicalutamide	3.488	452984	45638
9	Bicalutamide	3.712	537383	50538
10	Bicalutamide	3.535	617463	65483

Table 2: Accuracy results for Ezetamibe

%Concentration (at specification Level)	Area	Amount added(m)	Amount found(m)	% Recovey	Mean Recovery
50%	702873	5	5.10	101.8%	100.5%
100%	1390018	10	9.99	99.9%	
150%	2206281	15	14.9	99.1%	

Showing accuracy results for Simvastatin

%Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	239401	5	5.0	101.3%	100.0%
100%	480779	10	9.94	99.4%	
150%	733144	15	14.8	99.2%	

System suitability results for Ezetamibe (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	2158	1.8
2	1.0	2114	1.7
3	1.2	2069	1.7

System Suitability Results for Simvastatin

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	3536	1.7
2	1.0	2931	1.7
3	1.2	2713	1.7

Repeatability results of Simvastatin and Ezetamibe

	Peak name	RT	Area
1	Simvastatin	2.282	1313235
2	Simvastatin	2.312	1326776
3	Simvastatin	2.344	1347962
4	Simvastatin	2.351	1368872
5	Simvastatin	2.358	1363598
Mean			1344089
Std.dev			21267.38
%RSD			1.58229

	Peak name	RT	Area
1	Ezetamibe	3.433	46160

2	Ezetamibe	3.557	45294
3	Ezetamibe	3.623	44163
4	Ezetamibe	3.639	44079
5	Ezetamibe	3.704	43930
Mean			44725.2
Std.dev			865.8142
%RSD			1.935853

Intermediate precision for Ezetamibe

	Peak name	RT	Area
1	Simvastatin	2.381	1366825
2	Simvastatin	2.382	1379095
3	Simvastatin	2.384	1375825
4	Simvastatin	2.395	1364299
5	Simvastatin	2.412	1395271
6	Simvastatin	2.590	1393763
Mean			1379180
Std.dev			11950.85
%RSD			0.866519

Intermediate precision for Simvastatin

	Peak name	**RT**	**Area**
1	Ezetamibe	3.784	484545
2	Ezetamibe	3.797	484511
3	Ezetamibe	3.803	480804
4	Ezetamibe	3.845	485023
5	Ezetamibe	3.915	504952
6	Ezetamibe	4.607	485203
Mean			487506.2
Std.dev			7942.0
%RSD			1.6

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