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Cost Effective Stability Indicating Rapid, Efficient Analytical Method Development and Validation of Assay Method For Simultaneous Determination Of Simvastatin And Ezetimibe In Tablet Formulations

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ABSTRACT: A simple, rapid reverse phase high-performance liquid chromatographic assay method was developed and validated for the simultaneous estimation of Ezetimibe and Simvastatin in pharmaceutical dosage forms. Chromatography was carried out by using column Aquity BEH C-18, 100 x 2.1 mm, 1.7µ. Or equivalent internal diameter and flow rate .25 mL/min injection volume 2 µL, rum time 23 minutes was used with a mixture of mobile phasecontaining water and adjust pH 2.50 ± 0.05 with orthophosphoric acid. Filter and degass it. Determination of the different analytical parameters such as linearity, precision, accuracy, and specificity, filter equivalency and robustness was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The average recovery was found to be 100.2 and 101.8 for Ezetimibe and Simvastatin respectively. The proposed method is highly sensitive, precise and accurate, hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulations of Ezetimibe and Simvastatin.

KEY WORDS: Ezetimibe, Simvastatin, UPLC, Development, Validation, hyperlipidemic.

I. INTRODUCTION

Ezetimibe is a drug that lowers cholesterol. It acts by decreasing cholesterol absorption in the 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. Even though ezetimibe decreases cholesterol levels, the results of two major, high-quality clinical trials showed that it did not improve clinically significant outcomes, such as major coronary events, and actually made some outcomes, such as artery wall thickness, worse. Indeed, a panel of experts concluded in 2008 that it should "only be used as a last resort"[1-5].In one of those studies, a head-to-head trial in 2009, a much less expensive medication (extended-release niacin) was found to be superior. Ezetimibe actually increased the thickness of artery walls (a measurement of atherosclerosis) and caused more major cardiovascular events [6-7]. A more positive view of the benefits of Ezetimibe is offered by Britain's NICE statement which however was published in 2007 and may not have been updated to reflect the results of the above mentioned trials.Simvastatinis a hyperlipidemic drug used to control elevated cholesterol, or hypercholesterolemia. It is a member of the statin class of pharmaceuticals. Simvastatin is a synthetic derivate of a fermentation product of Aspergillus terreus. The drug is marketed under the trade name Zocor, as well as generically. The primary uses of simvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease. It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels[8].

The present study describes the development and validation of a new rapid, simple, sensitive and reproducible chromatographic method for the analysis of ezetimibe and simvastatin in tablet dosage form that offer certain advantages in its simplicity and sensitivity and applicable in routine analysis. It also describes the development of validation work as per ICH guidelines recommended by the Food and Drug Administration (FDA) of the United States[9-11].

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II. METHODOLOGY:

Instruments and Material: Following equipments were used for the validation studies.

Name of Instrument	Make & Model
UPLC	Waters 2489 dual wavelength
UPLC	Waters 2996 PDA detector
Balance	Mettler Toledo
pH meter	Metrohm

Working standard/Reference Standard:

Name of WS/RS	Potency (%)
Ezetimibe	99.3
Simvastatin	99.4

Reagents & solvents:

Name of Reagent/Solvent	Grade
Sodium dihydrogen phosphate monohydrate	AR grade
Orthophosphoric acid	AR grade
Acetonitrile	HPLC grade
Methanol	HPLC grade
Water	Milli Q or equivalent

Preparation of Buffer (Mobile Phase A): Take 1000 mL water and adjust pH 2.50 ± 0.05 with orthophosphoric acid. Filter and degass it.
Preparation of Mobile phase B: Use Acetonitrile as Mobile phase B.
Preparation of Buffer for diluent: Dissolve 1.36 gm of sodium dihydrogen phosphate monohydrate in 1000 mL water and mix.
Preparation of Diluent: Prepare a mixture of Acetonitrile, Methanol and Buffer in the ratio 30: 30: 40 v/v.
Preparation of Standard stock solution of Ezetimibe: Weigh accurately about 50 mg of Ezetimibe working standard into 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve. Dilute to volume with diluent and mix.
Preparation of Standard stock solution of Simvastatin: Weigh accurately about 50 mg of Simvastatin working standard into 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve. Dilute to volume with diluent and mix.
Preparation of Standard solution: Transfer 2 mL of Standard stock solution of Ezetimibe, 2 mL of Standard stock solution of Simvastatin in to 50 mL volumetric flask and dilute with diluent and mix.
Preparation of Sample Solution:Transfer 10 tablets into 200 mL volumetric flask. Add 10 mL water for complete dispersion of tablets then add 140 mL of diluent and sonicate in cool water for 30 minutes with intermittent shaking. Centrifuge the solution at 5000 rpm for 15 minutes. Further dilute 2 mL supernatant solution to 50 mL with diluent and mix. Filter the solution through 0.22µ PVDF filter and disregard first 5 mL filtrate.

Chromatographic Conditions:

Column	:	Aquity BEH C-18, 100 x 2.1 mm, 1.5µ. Or equivalent
Flow Rate	:	0.20 mL/min.
Detection	:	225 nm.

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Column Temperature	:	25˚C
Injection Volume	:	2.2µL
Run Time	:	25 minutes.

Gradient programme:

Time (mins)	Mobile phase A	Mobile phase B
0.0	70	30
1.0	50	50
6.0	50	50
7.0	35	65
18.0	35	65
18.2	70	30
23.0	70	30

III. RESULT & DISCUSSION:

Method development Study:

Selection of chromatographic method: Proper selection of chromatographic method depends on the nature of the drug,molecular weight, and solubility. Since ezetimibe and simvastatin is polar in nature, reverse phasechromatography has been used.

Development Trail 01: The mobile phase composition was mobile phase ammonium acetate buffer pH 5.0 and acetonitrile (30:70, v/v), Wavelength 230 nm, column C-18 (250*4.6 mm, 5µm) at a flow rate of 1.5 ml/min^{-1} in order to assess the impact of the acetonitrile content on the separation and chromatographic parameters like resolution, tailing factor and number of theoretical plates. Although increase of acetonitrile contents to 80 % reduced the retention time of simvastatin to 5.8 minutes and resolution between ezetimibe and simvastatin to about 5 but tailing was greater than 1.3 with less theoretical plates as compared to the plates obtained using optimum mobile phase composition (20:80, v/v buffer-acetonitrile).

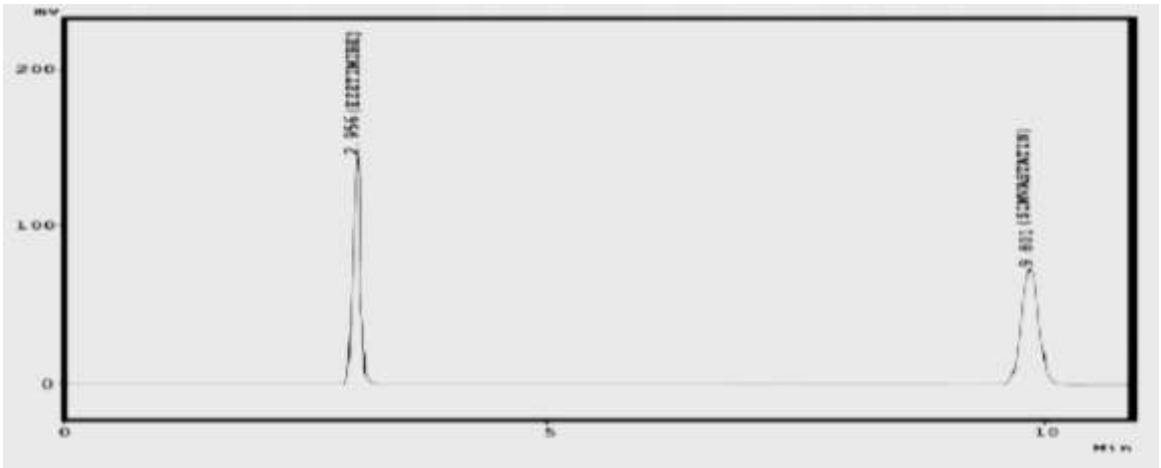

Figure 1. Chromatograms of ezetimibe and simvastatin in development trail 01.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 489-498 www.ijprajournal.com ISSN: 2249-7781

Development Trail 02: Various mobile phases were tried in different ratios for selection of mobile phase.The drug ezetimibe and simvastatin was injected with different mobile phase at different ratios with differentflow rates till a sharp peak without any interference peak containing spectrum was obtained.The different mobile phase were containing either one or the combinations of two or three offollowing solvents, acetonitrile, water, methanol, tetrahydrofuran.Tried at different ratios no favourable results obtained. But the mobile phasecontaining water and adjust pH 2.50 ± 0.05 with orthophosphoric acid. Filter and degass it. It gaveacceptable peak with retention time ezetimibe about 5 min and Simvastatin about 11 min

Water and adjust pH 2.50 ± 0.05 with orthophosphoric acid as mobile phase A and acetonitrile as mobile phase B was used with Column Aquity BEH C-18, 100 x 2.1 mm, 1.7µ. Or equivalent and flow rate .25 mL/min injection volume 2 µL and rum time 23 minutes was used in development trail 03 and shifted method in UPLC instead of HPLC for shorter run time. Gradient programme used for development trail 02.

Outcomes of Development Trails: In development trail 01 A mobile phase consisting acetate buffer pH 5.0 and acetonitrile at different ratios were tried to achievethe separation. But it was found that ezetimibe and simvastatin peak shape was not proper and less theoretical plate found.In next development trail method shifted in UPLC from HPLC with chromatographic conditions mentioned in development trail 02good symmetrical peaks were obtained. As our aim was to get good symmetrical peaks, this combination of chromatographic condition was selected for further study.

Method Validation Study:

Specificity: The retention time of the Ezetimibe and Simvastatin peaks in the chromatogram of the Sample preparation corresponds to that of the Ezetimibe and Simvastatin peaks in the chromatogram of the Standard preparation. Retention time and peak purity given in table 1 and figure 2.

Table 1: Table for Specificity

Sr. No.	Name	Ezetimibe		Simvastatin	
		PA	PT	PA	PT
2	Standard solution	0.130	1.088	0.170	1.221
4	Sample solution	0.140	1.077	0.200	1.108

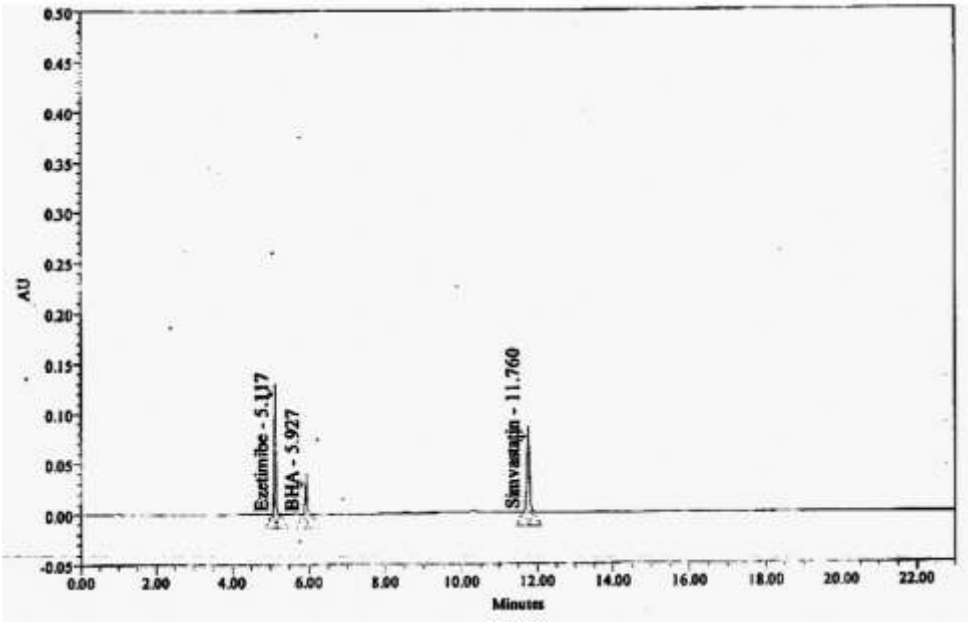

Figure 2: Standard solution – 10/10 mg

International Journal of Pharmaceutical Research and Applications
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Linearity and Range: A series of Standard preparations of Ezetimibe and Simvastatin was prepared over a range of 50% to 150% of the working concentration of Ezetimibe and Simvastatin in Ezetimibe and Simvastatin Tablets. (Minimum Five points should be in the range 80-120% of standard/ sample concentrationfor Assay). Since the working concentration is 10 µg per ml, 20 µg per ml of Ezetimibe, the range proposed is about 5 µg per ml to 30 µg per ml of Ezetimibe. Since the working concentration is 20, 40 and 80 µg per ml of Simvastatin, the range proposed is about 10 µg per ml to 120 µg per ml of Simvastatin. Linearity result given in table 2 and figure 3 &4.

Table 2: Table for Linearity-

Level	**Ezetimibe**		**Ezetimibe**	
	Concentration (µg/mL)	**Response (Area)**	**Concentration (µg/mL)**	**Response (Area)**
Lin – 1	5.00	81877	10.004	182274
Lin - 2	8.00	130609	16.006	287577
Lin - 3	10.00	166044	40.014	723969
Lin - 4	16.00	263184	64.023	1146401
Lin -5	18.00	297912	72.026	1297757
Lin -6	20.00	330346	80.029	1437427
Lin - 7	22.00	363715	88.032	1579849
Lin – 8	24.00	399865	96.035	1736462
Lin – 9	30.00	499118	120.043	2165602
Slope	16686		18017	
Intercept	-2299		-467	
Correlation	0.99996		0.99998	

International Journal of Pharmaceutical Research and Applications
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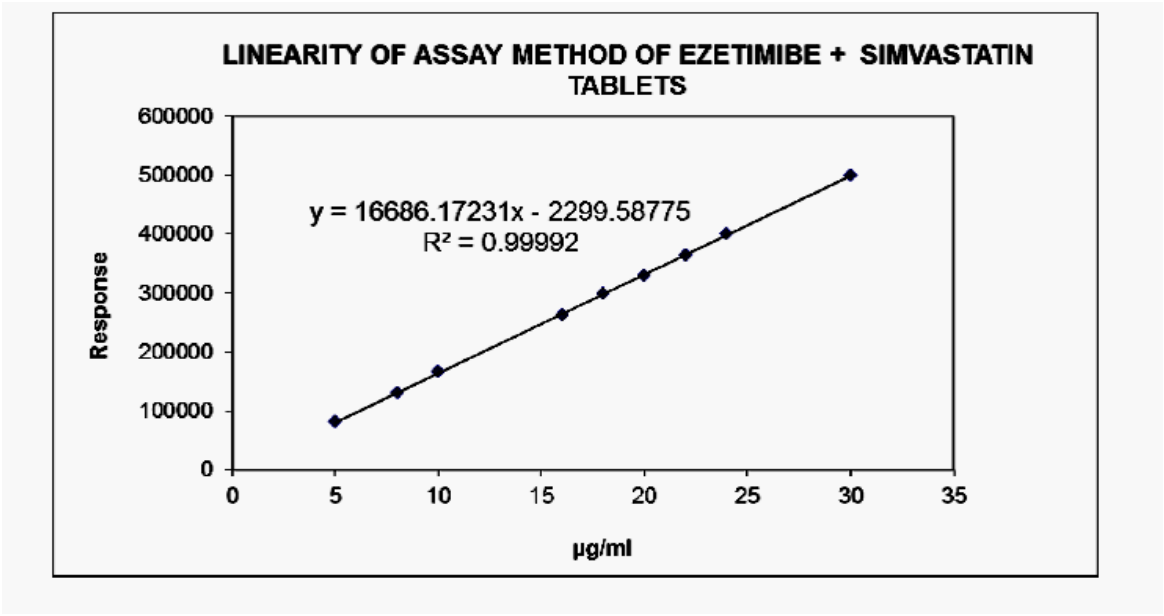

Figure3: Linearity graph –Ezetimibe

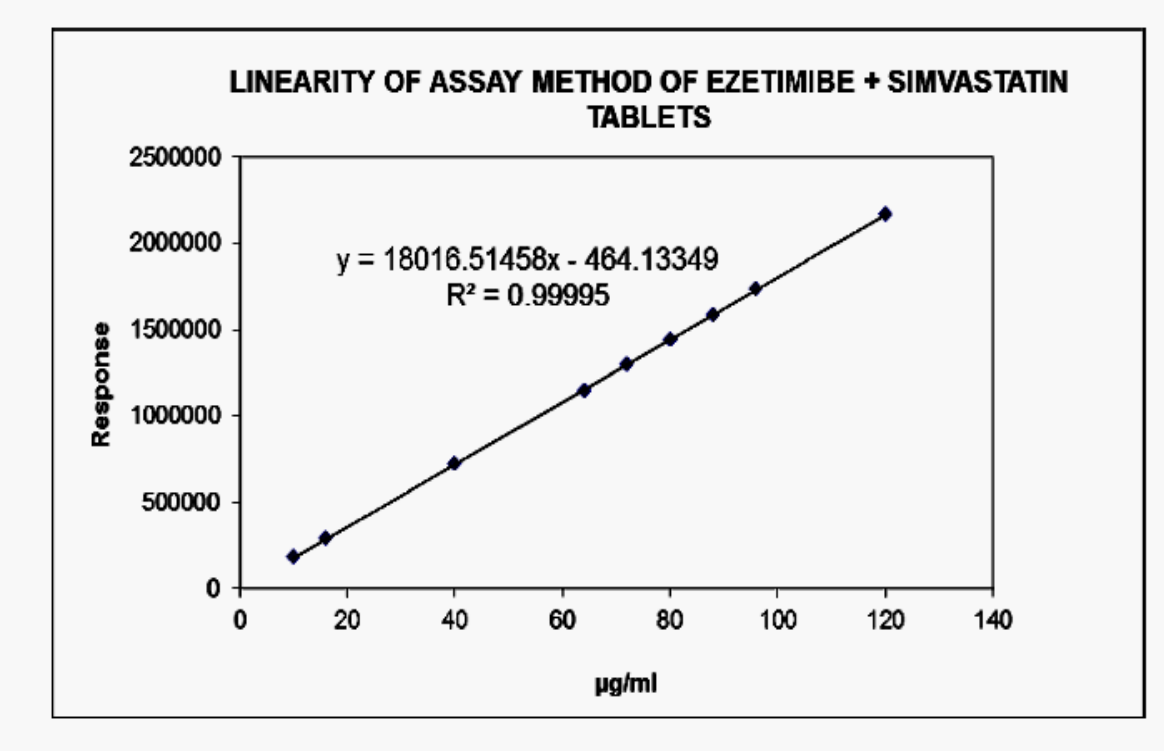

Figure 4: Linearity graph – Simvastatin

Accuracy (Recovery): Placebo of Ezetimibe and Simvastatin Tablets was spiked with Ezetimibe and Simvastatin at three different levels 80%, 100% and 120% of the label claim in triplicate. Results of accuracy mentioned in table no 3.

International Journal of Pharmaceutical Research and Applications
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Table 3: Table for Accuracy

Sample No.	% Recovery	
	Ezetimibe	Simvastatin
Sample solution-1	100.6	102.5
Sample solution-2	100.5	102.3
Sample solution-3	101.0	103.1
Acc.80%-1	99.7	101.2
Acc.80%-2	99.7	101.1
Acc.80%-3	99.7	101.2
Acc.100%-1	100.4	102.0
Acc.100%-2	100.5	102.1
Acc.100%-3	100.0	101.7
Acc.120%-1	100.0	101.5
Acc.120%-2	100.4	101.6
Acc.120%-3	100.2	101.7
Mean	100.2	101.8
SD	0.414	0.596
% RSD	0.41	0.59

PRECISION: Five replicate injections of the Standard preparation were made into the UPLC using the method as described under Methodology section. Precision result given in table 4.

Table 4: Table for System Precision

Injection	AreaEzetimibe	AreaSimvastatin
1	158594	1370930
2	158965	1372059
3	158936	1372857
4	158533	1370852
5	158422	1369714
Mean	158690	1371282
SD	245.881	1209.523
%RSD	0.15	0.09

Method Precision: Six sample preparations of Ezetimibe and Simvastatin Tablets were prepared and injected into the UPLC.The RSD is 0.13 % for Ezetimibe and 0.19 % for Simvastatin. Therefore, the UPLC method for the determination of Assay and preservative content in Ezetimibe and Simvastatin Tablets is reproducible.

Ruggedness(Intermediate Precision): Six sample preparations of the same lot of Ezetimibe and Simvastatin Tablets were by a different analyst, using different column on a different day and injected in duplicate into a different UPLCalong with Standard preparation. Result are given in table no 5.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 489-498 www.ijprajournal.com ISSN: 2249-7781

Table 5: Table for Ruggedness

Sample	**Ezetimibe**		**Simvastatin**	
	Analyst -1	Analyst -2	Analyst -1	Analyst -2
Mean	104.9	101.6	100.7	97.3
SD	0.137	0.486	0.194	0.413
%RSD	0.13	0.48	0.19	0.42
Overall Mean	103.3		99.0	
Overall SD	1.774		1.793	
Overall %RSD	1.72		1.81	

Robustness: The samples along with standard will be injected in duplicate under different chromatographic conditions. Results obtained in robustness parameter of different condition are mentioned in table no. 6 and table no 7.

Table 6: Table for Change in pH of Buffer

	Control	**Ezetimibe**		**Simvastatin**	
		(+0.2 units)	**(-0.2 units)**	**(+0.2 units)**	**(-0.2 units)**
	101.0	100.6	102.0	97.7	97.9
	101.2	102.3	102.5	97.6	97.8
	101.7	101.4	102.4	97.3	97.5
Mean	101.3	101.4	102.3	0.608	0.608
SD	0.361	0.85	0.265	0.62	0.62
%RSD	0.36	0.84	0.26	96.6	96.8

Table 7: Table for Change in Flow rate:

	Control	Ezetimibe		Simvastatin	
		(+0.02 ml)	(-0.02 ml)	(+0.02 ml)	(-0.02 ml)
	101.0	100.9	102.4	96.7	97.6
	101.2	103.5	104.3	97.2	98.0
	101.7	102.0	102.7	97.8	98.4
Mean	101.3	102.1	103.1	97.2	98.0
SD	0.361	1.305	1.021	0.551	0.400
%RSD	0.36	1.28	0.99	0.57	0.41

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 489-498 www.ijprajournal.com ISSN: 2249-7781

Filter Equivalency: Result of filter equivalency is given in table no 8.

Table 8: Table for Filter Equivalency -

Sr No.	Ezetimibe			Simvastatin		
	Centrifuge	Teflon	PVDF	Centrifuge	Teflon	PVDF
1	105.8	106.1	106.3	101.0	101.2	101.3
2	106.0	106.1	106.8	101.1	101.3	101.8
3	105.7	106.2	106.6	100.9	101.4	101.6
Mean	105.8	106.1	106.6	101.0	101.3	101.6
% Correlation with centrifuge	--	100.3	100.8	--	100.3	100.6

IV. SUMMARY AND CONCLUSION:

A stability indicating UPLC method has been developed for the assay of ezetimibe and Simvastatin Tablets. In Specificity the Ezetimibe and Simvastatin peaks are pure in Standard solution and Sample solution.No interference was observed from Blank and Placebo at the retention time of Ezetimibe and Simvastatin peak. Also, The Ezetimibe and Simvastatin is pure in Standard solution and Sample solution. Therefore, the UPLC method for the determination for Assay of Ezetimibe and Simvastatin Tablets is specific. In Linearity Correlation coefficient is more than 0.999. In Accuracy Mean recovery for Ezetimibe is 100.2 % & RSD is 0.41 %. Mean recovery for Simvastatin is 101.8 % & RSD is 0.59 %. Therefore, the UPLC method for the determination for Assay of Ezetimibe and Simvastatin Tablets is accurate.The RSD of system precision is 0.15 % for Ezetimibe, 0.09 % for Simvastatin. Therefore, the UPLC method for the determination of Assay isprecise and rugged. The Mean Filtration Recovery is within limits for Teflon 0.45µ and 0.22µ PVDF membrane filter. Therefore, Teflon 0.45µ and 0.22µ PVDF filter is suitable for filtration of samples in the UPLC method for the determination forAssay of Ezetimibe and Simvastatin Tablets.

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REFERENCES:

[1]. Goel A, Baboota S, Sahni JK,Srinivas S, Gupta RS, Gupta A, Semwal VP, Ali J. Development and Validation of Stability-Indicating Assay Method by UPLC for a Fixed Dose Combination of Atorvastatin and Ezetimibe. Journal ofChromatographic Science. 2013; 51: 222–228.

[2]. Malenovic D, Ivanovic M, Medenica B, Jancic S, Markovic. HPLC determination of ezetimibe and simvastatin in pharmaceutical formulations Journal of Separation Science. 2014; 27: 1087-1095.

[3]. Paulo RO, Thiago B, Vitor T, Sergio LD. Simultaneous liquid chromatographic determination of ezetimibe and simvastatin in pharmaceutical products. Journal of AOAC International. 2007; 90:1566-1572.

[4]. MelaniL,MillsR, HassmanD.Efficacy and safety of ezetimibe co-administered with pravastatin in patients with primary hypercholesterolemia: A prospective, randomized, double-blind trial, European Heart Journal. 2003; 24:717-728.

[5]. Kadav AA, Vora DN. Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets, Journal of Pharmaceutical and Biomedical Analysis. 2008; 48: 120-126.

[6]. Novakova L, SatinskyD. HPLC methods for the determination of simvastatin and atorvastatin, Trends in Analytical Chemistry. 2008; 27: 352-367.

[7]. Seshachalam U, Kothapally CB. HPLC analysis for simultaneous determination of atorvastatin and ezetimibe in pharmaceutical formulations. Journal of Liquid Chromatography and Related Technology. 2008; 31:714–721.

[8]. Chavhan V, Reddy K, Ahhirao K. Development of UV spectrophotometric methods and validation for estimation of simvastatin in bulk and tablet dosage form by absorbance maxima and area under the curve method. Journal of Applied Pharmaceutical Science. 2014; 6: 55–64.
[9]. Erturk S, AktasES, Ersoy L. A HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. Journal of Pharmaceutical and Biomedical Analysis.2003; 33:1017-1023.
[10]. Center for Drug Evaluation and Research, US Food and Drug Administration. Reviewer Guidance, Validation of Chromatographic Methods. FDA; Rockville, MD, USA: 2017.
[11]. International Conference on Harmonization, ICH harmonized tripartite guideline validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, 2005.