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A NEW RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF EZETIMIBE AND SIMVASTATIN IN BULK AS WELL AS IN PHARMACEUTICAL FORMULATION BY USING PDA DETECTOR

Sowjanya Surisetti*[1], S. Ashutosh Kumar[2], J. Saravanan[3], Manidipa Debnath[4], V. Greeshma[5], N. Sai Krishna[6], G. Satya Sri[7]

[1,2&7]Department of Pharmaceutical Analysis & Quality Assurance, A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, A.P

[3,5&6]Department of Pharmacology, A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, A.P

[4]Department of Pharmaceutics, A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, A.P

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***Correspondence for Author:**
S. Ashutosh Kumar,
Associate Professor,
Department of Pharmaceutical Analysis & Quality Assurance,
A.K.R.G College of Pharmacy,
Nallajerla, West Godavari,
534112, A.P, India.
ashu.mpharm2007@gmail.com

ABSTRACT

Ezetimibe is a drug that lowers cholesterol. It acts by decreasing cholesterol absorption in the intestine. Ezetimibe decreases cholesterol levels, the results of two major, high-quality clinical trials (in 2008 and 2009) 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. Ezetimibe actually increased the thickness of artery walls (a measurement of atherosclerosis) and caused more major cardiovascular events. Simvastatin is a hypolipidemic 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. This study was designed to develop and validate a simple, sensitive, precise, and specific reverse phase high-performance liquid chromatographic (HPLC) method for the determination of Ezetimibe and simvastatin in bulk and its tablet dosage forms. The HPLC separation was carried out by reverse phase chromatography on XTerra

column C18 (4.6 x 150mm, 3.5 µm) with a mobile phase composed sodium dihydrogen ortho phosphate [the pH was adjusted to 3.0 by using Orthophosporic Acid] & Acetonitrile in the ratio of 30:70 v/v in isocratic mode at a flow rate of 1.0 ml/min. The run time was maintained 8 mins. The retention times obtained for the drug Ezetimibe was around 2.2mim and for the drug simvastatin was 3.2. The detection was monitored at 242 nm. The calibration curve was linear from 50 to 90µg/ml. The inter-day and intra-day precision was found to be within the specified limits. The proposed method has adequate sensitivity, reproducibility, and specificity for the determination of Ezetimibe and Simvastatin in bulk and its tablet dosage forms. The limit of detection for the drug Ezetimibe and Simvastatin was found to be 0.072µg/ml and 0.12µg/ml solution. The Limit of Quantification for the drug Ezetimibe and Simvastatin was found to be 0.15µg/ml and 0.41µg/ml respectively. The Accuracy recoveries were 98.0-102.0% and reproducibility was found to be satisfactory. The proposed method is simple, fast, accurate, and precise for the quantification of Ezetimibe and Simvastatin in the dosage form, bulk drugs as well as for routine analysis in quality control. The present work was undertaken with the aim to develop and validate a rapid and consistent RP-HPLC method in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, Accurate and Precise method for the Quantification of drug in the dosage form, bulk drug as well as for routine analysis in Quality control. Overall the proposed method was found to be suitable and accurate for the Quantitative determination in Bulk as well as in Pharmaceutical dosage form.

Keywords: High Performance Liquid Chromatography; Sodium Dihydrogen ortho Phosphate; Acetonitrile; Ezetimibe and Simvastatin.

INTRODUCTION

Ezetimibe [(3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one] is a drug that lowers cholesterol. It acts by decreasing cholesterol absorption in the intestine. It may be used alone (marketed as Zetia or Ezetrol), when other cholesterol lowering medications are not tolerated, or together with statins (e.g., Ezetimibe/simvastatin, marketed as Vytorin and Inegy) when statins alone do not control cholesterol. Even though Ezetimibe decreases cholesterol levels, the results of two major, high-quality clinical trials (in 2008 and 2009) 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". 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 (Fig. No.1) actually increased the thickness of artery walls (a measurement of atherosclerosis) and caused more major cardiovascular events. 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. Ezetimibe localises at the brush border of the small intestine, where it inhibits the absorption of cholesterol from the intestine. Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tract epithelial cells as well as in hepatocytes. In addition to this direct effect, decreased cholesterol absorption leads to an up regulation of LDL-receptors on the surface of cells and an increased LDL-cholesterol uptake into cells, thus decreasing levels of LDL in the blood plasma which contribute to atherosclerosis and cardiovascular events. Common adverse drug reactions (≥1% of patients) associated with Ezetimibe therapy are headache and/or diarrhea (steathorrea). Infrequent adverse effects (0.1–1% of patients) include: myalgia and/or raised liver function test (ALT/AST) results. Rarely (<0.1% of patients), hypersensitivity reactions (rash, angioedema) or myopathy may occur. Side effects include gastro-intestinal disturbances; headache, fatigue; myalgia; rarely arthralgia, hypersensitivity reactions (including rash, angioedema, and anaphylaxis, hepatitis; very rarely pancreatitis, cholelithiasis, cholecystitis, thrombocytopenia, raised creatine kinase, myopathy, and rhabdomyolysis.

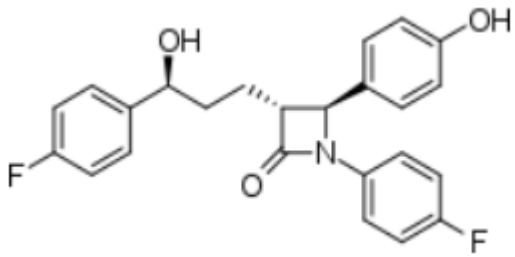

Fig. No. 1 Chemical Structure of Ezetimibe

Simvastatin [(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] is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia (Fig. No.2). 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. Common side effects (>1% incidence) may include abdominal pain, diarrhea, indigestion, and a general feeling of weakness. Rare side effects include joint pain, memory loss, and muscle cramps. Cholestatic hepatitis, hepatic cirrhosis, rhabdomyolysis and myositis have been reported in patients receiving the drug chronically [1-17] The drug is officially listed in 2004 United States Pharmacopoeia and the official method of its determination is UV-spectrophotometry, and various other methods are HPTLC, miscellar eletrokinetic chromatography and voltammetry have been reported for the assay of SMT in pharmaceuticals . The method development bottleneck result from the requirement to generate a quantitative and qualitative profile of impurities, enabling the reporting of the identity of each chemical moiety. Two official methods utilising HPLC GRA DIENT methodology are reported in European Pharmacopoeia (EP) and United State Pharmacopoeia (USP) [18-24].

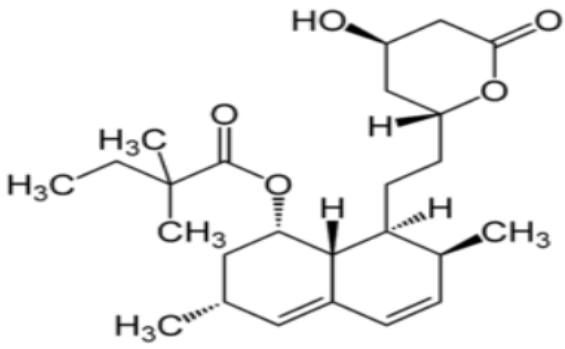

Fig. No.2 Chemical Structure of Simvastatin

METHOD DEVELOPMENT FOR EZETIMIBE & SIMVASTATIN

Chemicals and Reagents Used

The following chemicals were procured for the process: Water [HPLC Grade], Methanol [HPLC Grade], Ezetimibe & Simvastatin [Working standards] & Orthophosphoric acid all the chemicals were procured from STANDARD SOLUTIONS and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions

Equipment	: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column	: X terra C18 (4.6 x 150mm, 3.5µm, Make: ACE)
Mobile phase	: Phosphate Buffer [70%] & Acetonitrile HPLC Grade [30%]
Flow rate	: 1.0 mL per min
Wavelength	: 242 nm
Injection volume	: 30 µl
Column oven	: Ambient
Run time	: 8min
Detector	: Photo diode array
Soft ware	: Empower 2
Retention Time	: Ezetimibe 2.2min & Simvastatin 3.2min.

Preparation of Sodium Phosphate buffer [25]: The buffer solution was prepared by accurately weighing 2.5milligrams of Sodium di hydrogen ortho phosphate and transferred into a clean, dry 1000ml volumetric flask, dissolved and diluted upto 1000ml with water [HPLC Grade]. The pH was adjusted to 6.0 with Orthophosporic acid.

Preparation of mobile phase: The mobile phase was prepared by mixing above buffer 300 mL (30%) and 700 mL of Acetonitrile [HPLC Grade] (70%) and degassed in ultrasonic water bath for 5 minutes. It was filtered through 0.45 µ membrane filter under vacuum filtration.

Preparation of Diluent: The Mobile phase was used as Diluent.

Preparation of the Ezetimibe & Simvastatin Standard & Sample solution

Preparation of Standard Solution: The standard solution was prepared by accurately weighing 10 mg of Ezetamibe & Simvastatin [working standard] and transferred into a 10ml clean and dry volumetric flask. About 7 ml of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution pipette out 0.8 ml of Ezetamibe & Simvastatin into a 10ml volumetric flask and diluted up to the mark with the diluent.

Preparation of Sample Solution: The sample solution was prepared by accurately weighing 10 mg of Ezetamibe & Simvastatin [sample] and transferred into a 10ml clean and dry

volumetric flask. About 7mL of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared stock solution pipette out 0.8ml of Ezetamibe & Simvastatin into a 10ml clean and dry volumetric flask and the volume was made upto the mark with the diluent. About 20 µL of the standard & sample were injected into the chromatographic system and measured the areas for the Ezetamibe & Simvastatin peaks and calculated the %Assay by using suitable formulae.

System Suitability [26-28]

The Tailing factor for the peaks due to Ezetamibe & Simvastatin in Standard solution should not be more than 1.5

The Theoretical plates for the Ezetamibe & Simvastatin peaks in Standard solution should not be less than 2000.

Calculation for Ezetamibe

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt.}{Label\ Claim} \times 100$$

Where

AT = Average area counts of sample preparation.

AS = Average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT =Weight of test taken in mg.

DS =Dilution of standard solution

DT =Dilution of sample solution

P = Percentage purity of working standard

System Suitability Results for Ezetamibe

1) The Tailing factor obtained from the standard injection was **1.32.**

2) The Theoretical Plates obtained from the standard injection was **2761.**

Assay Result for Ezetamibe

$$\frac{921493}{884127} \times \frac{10}{100} \times \frac{0.8}{10} \times \frac{10}{77.5} \times \frac{10}{0.8} \times \frac{99.9}{100} \times \frac{74.5}{100} \times 100 = 100.09\%$$

Calculation for Simvastatin

$$\textbf{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt.}{Label\ Claim} \times 100$$

Where:

AT = Average area counts of sample preparation.

AS = Average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT = Weight of test taken in mg.

DS = Dilution of standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

System Suitability Results for Simvastatin:

1) The Tailing factor obtained from the standard injection was **1.32**

2) The Theoretical Plates obtained from the standard injection was **2761.**

Assay Results for Simvastatin

$$\frac{1041937}{1013084}\times\frac{10}{10}\times\frac{0.8}{10}\times\frac{10}{77.5}\times\frac{10}{0.8}\times\frac{74.5}{10}\times\frac{99.9}{100}\times 100 = 100.40\%$$

VALIDATION DEVELOPMENT [29-36]

1. Precision: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The %RSD for the area of five replicate injections was found to be within the specified limits.(Table No.1)

Table No.1: The Precision results were summarized for the drugs Ezetamibe & Simvastatin.

Sr. No.	INJECTION	EZETIMIBE AREA	SIMVASTATIN AREA
1	Injection-1	882524	1018279
2	Injection-2	881215	1016821
3	Injection-3	882709	1019092
4	Injection-4	882812	1018954
5	Injection-5	883364	1019187
	Average	882525	1018467
	Standard Deviation	796.1	986.3
	%RSD	0.09	0.10

Acceptance Criteria: The % RSD for the area of all the five standard injections should not be more than 2%.

2. Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. (Table No.2)

Table No.2: The Ruggedness results were summarized for the drugs Ezetamibe & Simvastatin.

Sr. No.	Injection	Ezetimibe Area	Simvastatin Area
1.	Injection-1	882222	1017321
2.	Injection-2	883098	1018064
3	Injection-3	883441	1017142
4.	Injection-4	882989	1017653
5.	Injection-5	885032	1019474
	Average	883356	1017931
	Standard Deviation	1037.2	931.4
	%RSD	0.12	0.09

Acceptance Criteria: The % RSD for all the five standard injections results should not be more than 2%

3. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solution was injected with Accuracy -50%, 100% and 150% solutions. The amount found was calculated and amount added for Ezetamibe & Simvastatin was estimated. The individual recovery and mean recovery values were also calculated (Table no.3 & 4).

Table No.3: The Accuracy result was summarized for the drug Ezetamibe.

Sr. No.	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1.	50%	928662	5.0	4.95	99.03%	100.15%
2.	100%	1909125	10.0	10.18	101.8%	
3.	150%	2802211	15.0	14.94	99.6%	

Table No.4: The Accuracy result was summarized for the drug Simvastatin.

Sr. No.	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1.	50%	1046630	5.0	4.96	99.2%	99.62%
2.	100%	2133903	10.0	10.12	101.1%	
3.	150%	3114111	15.0	14.77	98.44%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

4. Linearity: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. A graph was plotted for peak area versus concentration and the correlation coefficient was calculated. (Table No.5)

Table No.5: Linearity results for the drug Ezetamibe & Simvastatin.

Sr. No.	Linearity Level	Concentration	Ezetamibe Area	Simvastatin Area
1	I	50µg/ml	717289	820444
2	II	60 µg/ml	814897	929435
3	III	70 µg/ml	897354	1025070
4	IV	80 µg/ml	1000043	1142674
5	V	90 µg/ml	1088803	1240551
Correlation Coefficient			0.9996	0.996

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

5. Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

a. Limit of Detection of Ezetamibe

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 49 µV

Signal Obtained from LOD solution (3.9% of target assay concentration) : 154 µV

S/N = 154/49 = 3.14

b. Limit of Detection of Simvastatin

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 49 µV

Signal Obtained from LOD solution (3.9% of target assay concentration) : 151 µV

S/N = 151/49 = 3.08

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

6. Limit of Quantification: The Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/ or degradation products. Several approaches for determining the Quantification limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

a. Limit of Quantification of Ezetamibe

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOQ solution (6.5% of target assay concentration) : 518µV

S/N = 518/49 = 10.57.

b. Limit of Quantification of Simvastatin

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOQ solution (6.5% of target assay concentration) : 521µV

S/N = 521/52 = 10.01

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

7. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a. Variation at flow rate (0.9 ml/min to 1.1ml/min): The Standard solution of Ezetamibe & Simvastatin was prepared and analysed using the various flow rates along with actual flow rate (Table no. 6 & 7).On evaluation of the obtained results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it was indicated that the method was robust even by change in the flow rate ±10%.

Table No.6: The Robustness result was summarized for the drug Ezetamibe

Sr. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2198	1.5
2	1.0	2026	1.39
3	1.1	1983	1.8

Table No.7: The Robustness result was summarized for the drug Simvastatin

Sr. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2926	1.5
2	1.0	2761	1.32
3	1.1	2631	1.4

b. The Organic composition in the Mobile phase varied from 20% to 40%. The Standard solution of Ezetamibe & Simvastatin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method (Table No.9 & 10). On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase did not affect the method significantly. Hence it was indicated that the method was robust even by change in the Mobile phase ±10.

Table No.8The System suitability result summarized for the drug Ezetamibe

Sr. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2056	1.8
2	Actual	2026	1.39
3	10% more	2186	1.8

Table No.9The System suitability result summarized for the drug Simvastatin

Sr. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2186	1.8
2	Actual	2761	1.32
3	10% more	2968	1.5

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precised, accurate RP-HPLC method for the analysis of Ezetamibe & Simvastatin in pharmaceutical dosage forms. In order to effect the separation of the drug under isocratic conditions, mixtures of Phosphate Buffer [with different pH] and Acetonitrile [HPLC Grade] in different combinations were tested as mobile phase on a XTerra column C18 (4.6 x 150mm, 3.5 µm). The mobile phase composed of sodium dihydrogen ortho phosphate [the pH was adjusted to 3.0 by using Orthophosporic Acid] & Acetonitrile in the ratio of 30:70 v/v in isocratic mode at a flow rate of 1.0 ml/min was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The run time was maintained 8 mins. The retention times obtained for the drug Ezetimibe was around 2.2mim and for the

drug simvastatin was 3.2min. A model chromatogram showing the separation of Ezetamibe & Simvastatin was represented in Fig. no.3.

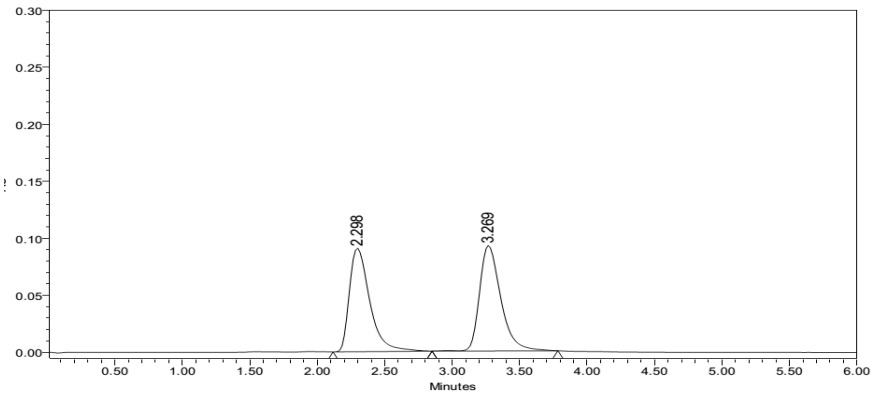

Fig. no. 3The Chromatogram representing the separation of Ezetamibe & Simvastatin

For Precision Studies the standard solution was injected for five times and measured the area for all the five injections in RP-HPLC system. The % RSD for the area of five standard injections results should not be more than 2%**.**The %RSD for the area of the all five standard injections of both the drugs was found to be within the limits. So, that the method was said to be precise. The data was represented in table no. 1 and the chromatogram was represented in Fig. No. 4

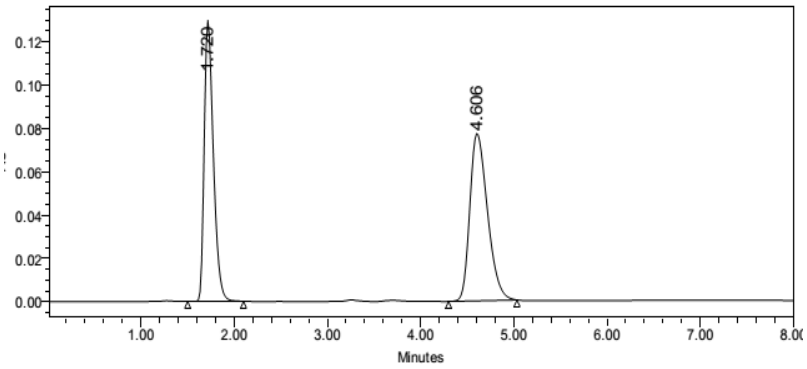

Fig. no. 4The Chromatogram representing for Precision

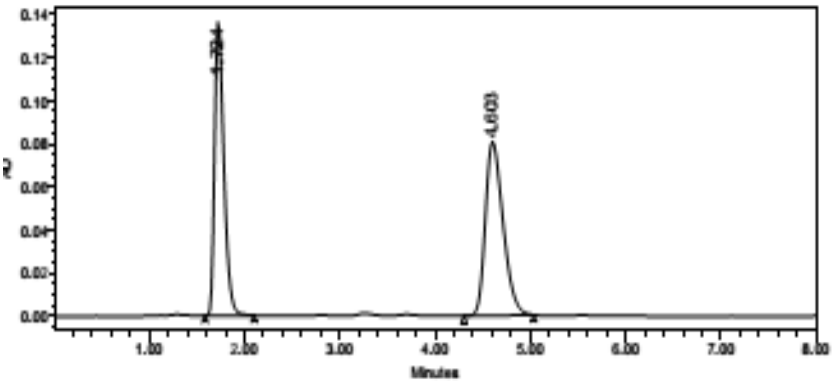

Fig. no. 5The Chromatogram representing for the Sample

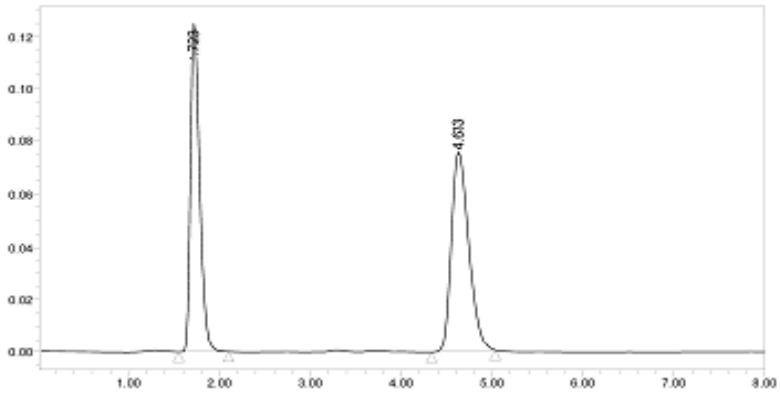

Fig. no. 6The Chromatogram representing for the Standard

When the drugs Ezetamibe & Simvastatin were analyzed by the proposed method for intra and inter-day (Ruggedness) variation results, a low coefficient of variation was observed (Table 2). This showed that the present HPLC method was highly precise and it is represent in Fig no. 7. The % RSD for the area of five standard injections results were not more than 2%.

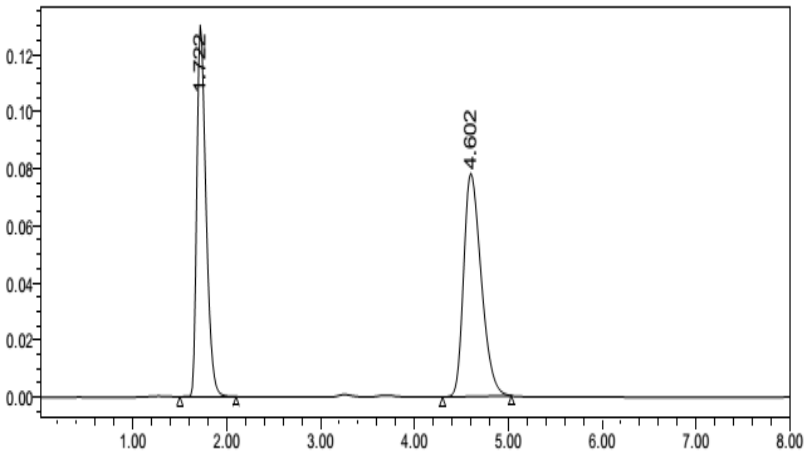

Fig. No. 7The Chromatogram representing for Ruggedness

For Accuracy, th**e** standard solutions with different concentrations were injected along with Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. The amount found was calculated & the amount added for Ezetamibe & Simvastatin was calculated. The individual recovery and mean recovery values were also calculated. The data were represented in Table 3 & 4. A model chromatogram was represented in Fig.No.8, 9 & 10.

The % Recovery for each level was found to be in between 98.0 to 102.0%. The % recovery for each level was found to be within the limits. So, that the method was said to be accurate.

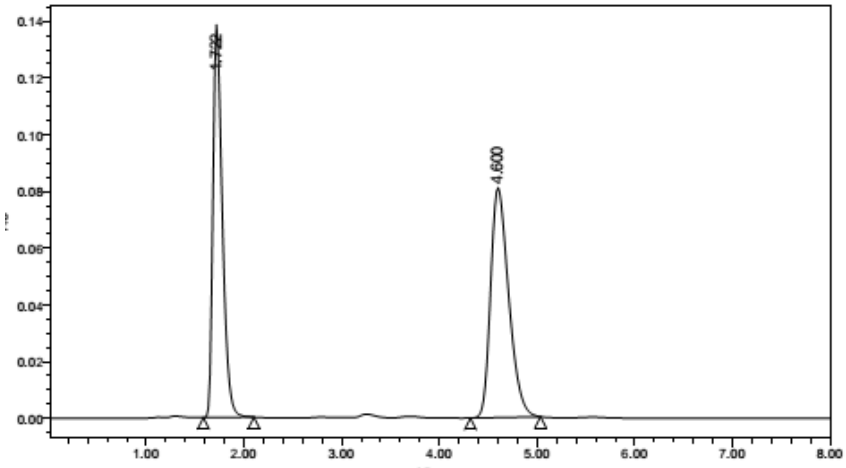

Fig. No. 8The Chromatogram representing for Accuracy 50%

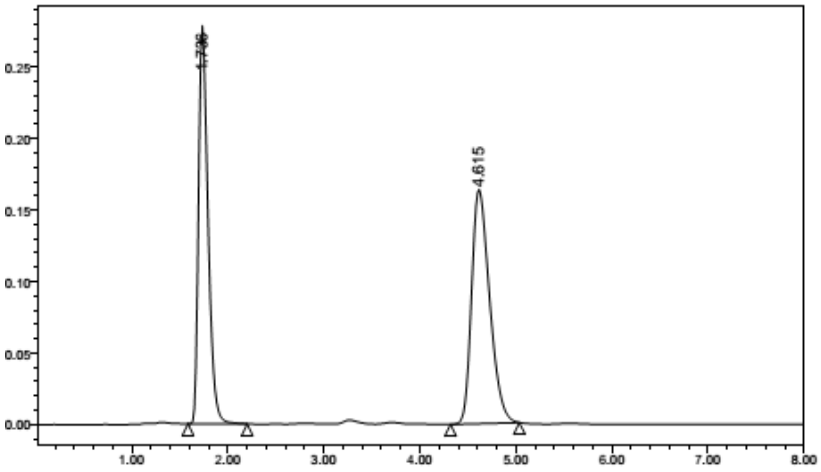

Fig. No. 9The Chromatogram representing for Accuracy 100%

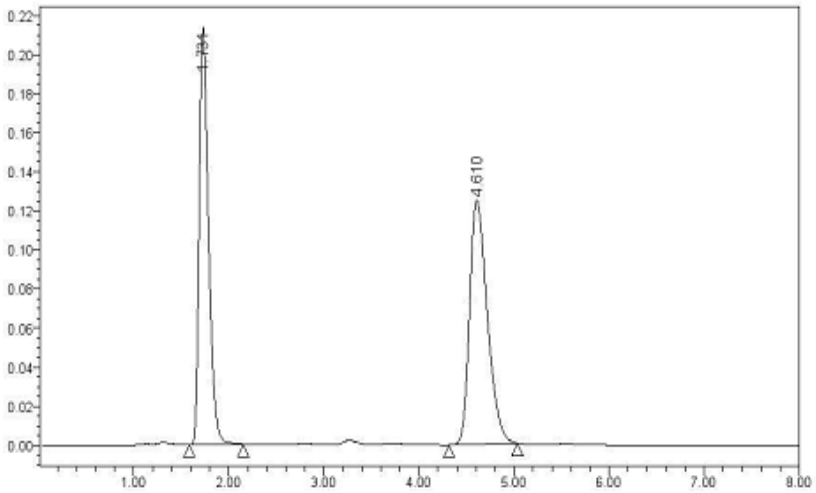

Fig. No. 9The Chromatogram representing for Accuracy 150%

In order to test the linearity of the method, the solutions of each level were injected into the chromatographic system and measured the peak area. A graph was plotted, Peak area versus Concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated. The data was represented in table 5. The Correlation coefficient was found 0.999.The correlation coefficient was found to be within limits. So, that the method was said to be linear. The Linearity Curve was represented in Fig. No.10 & 11.

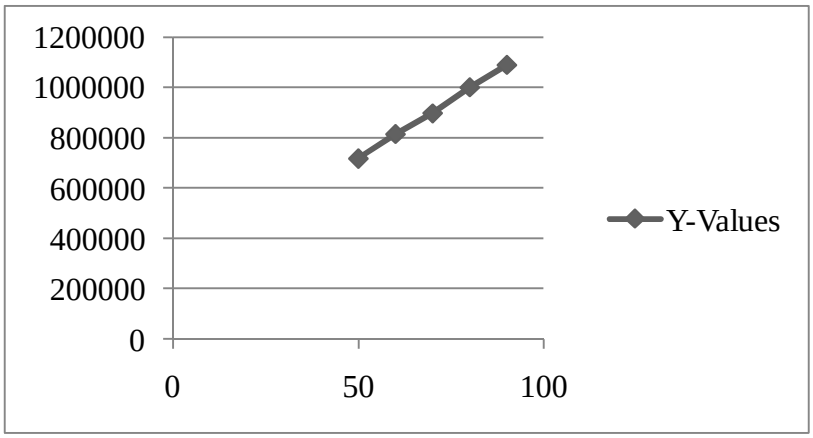

Fig. No. 10The Chromatogram representing Linearity Curve for Ezetamibe

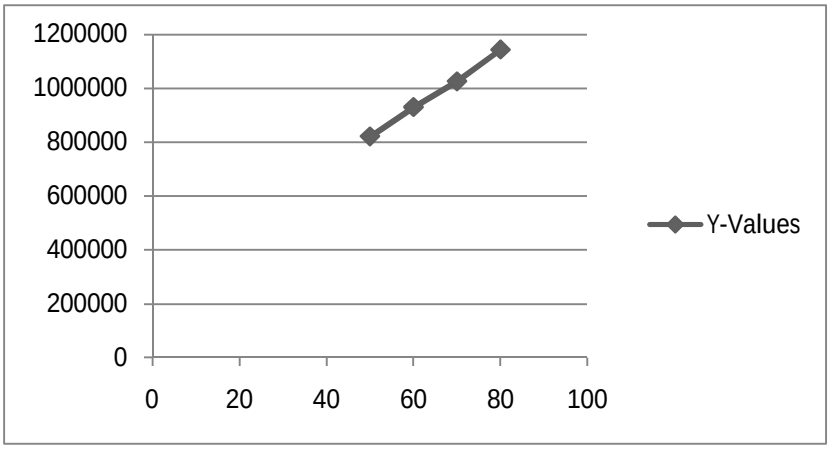

Fig. No. 11The Chromatogram representing Linearity Curve for Simvastatin

Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The limit of detection for the drug Ezetimibe and Simvastatin was found to be 0.072µg/ml and 0.12µg/ml solution. The Limit of

Quantification for the drug Ezetimibe and Simvastatin was found to be 0.15µg/ml and 0.41µg/ml respectively. A model chromatogram was represented in Fig. no. 12 & 13.

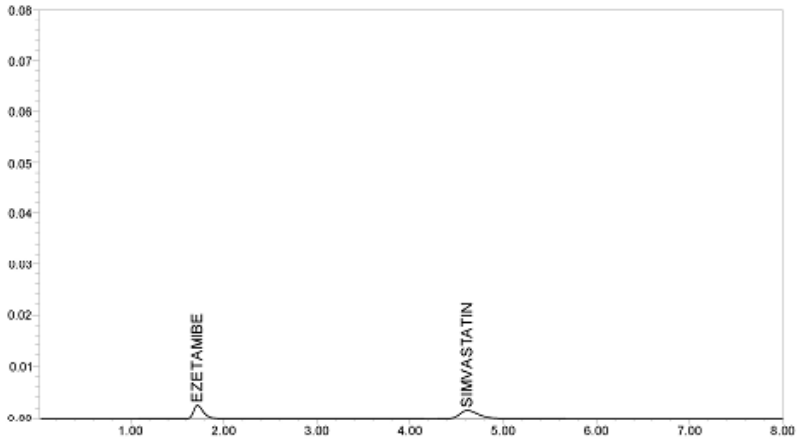

Fig. No. 12The Chromatogram representing for LOD

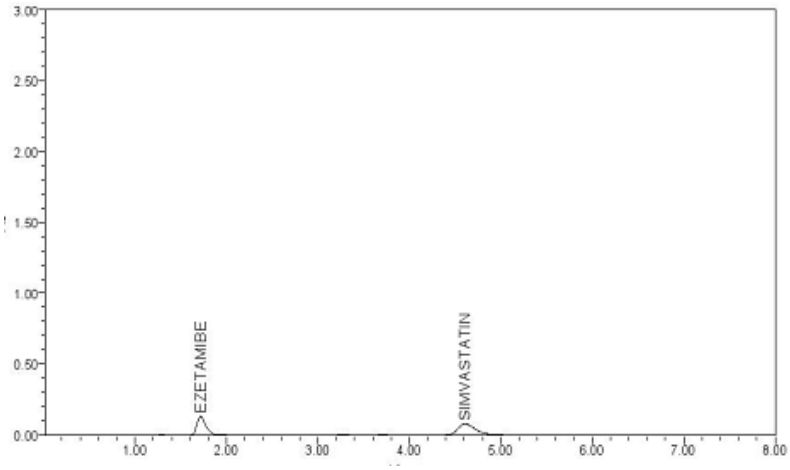

Fig. No. 13The Chromatogram representing for LOQ

Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate buffer and Acetonitrile in the mobile phase. On evaluation of the above results, it can be concluded that the variation in 10% organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in mobile phase ±10%.The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table no.6, 7, 8 & 9 and chromatograms were represented in Fig. no. 14, 15, 16 &17.

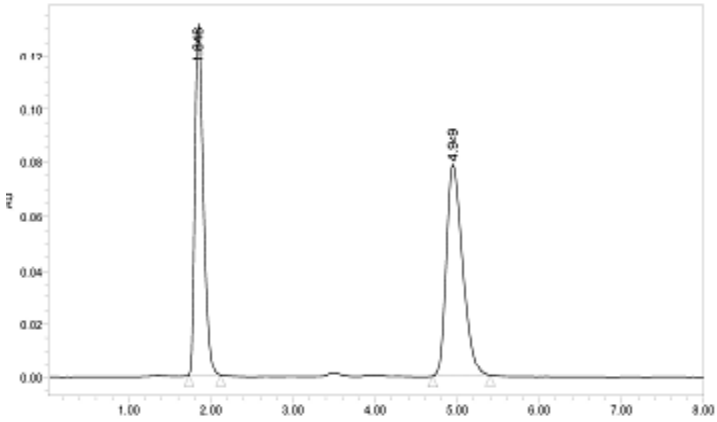

Fig. No. 14The Chromatogram representing for Robustness (Less Flow Rate)

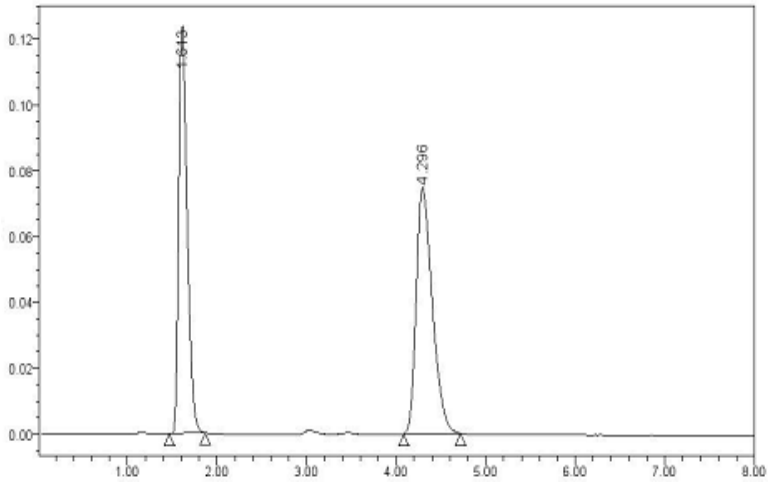

Fig. No. 15The Chromatogram representing for Robustness (More Flow Rate)

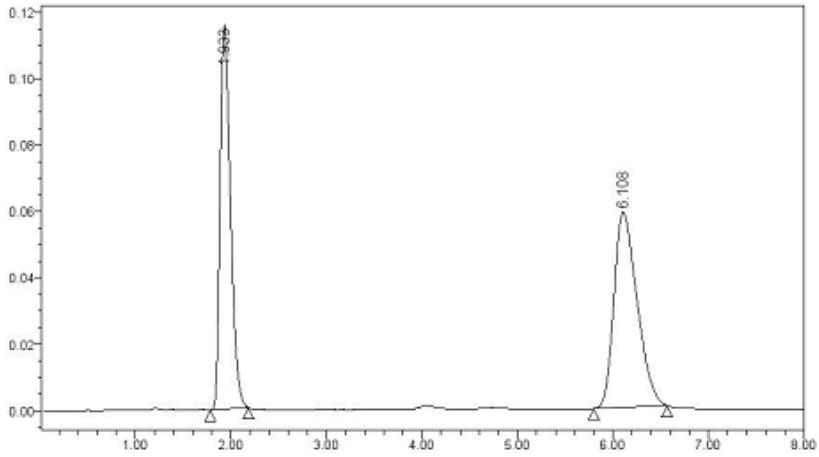

Fig. No. 16The Chromatogram representing for System Suitability (Less Organic Phase Composition in mobile phase)

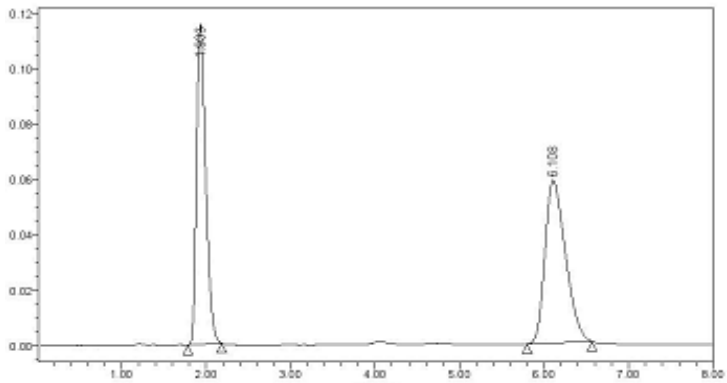

Fig. No. 17The Chromatogram representing for System Suitability (More Organic Phase Composition in mobile phase)

CONCLUSION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage is important in pharmacokinetic, toxicological biological studies. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. A simple RP-HPLC method was developed for the determination of Ezetimibe and Simvastatin, in pharmaceutical dosage form. A X terra C18 (4.6 x 150mm, 3.5µm) column from Waters in isocratic mode, with mobile phase composed with Phosphate Buffer [pH 6.0] and Acetonitrile. The flow rate was 1.0ml/min and effluent was monitored at 242nm and retention times were 2.2 and 3.2 min. As per ICH guidelines the method was validated over the range of 10-1000ppm and was accurate (average accuracies of three different concentrations ranged from 50%, 100% and 150%) and precise. The proposed method can be used as alternative methods to the reported ones for the routine determination of selected drugs under the study in pharmaceutical dosage forms. Thus the purpose of the present investigation was successfully achieved.

Hence it was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Ezetimibe and Simvastatin in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be

superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Ezetimibe and Simvastatin depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Ezetimibe and Simvastatin. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Ezetimibe and Simvastatin from tablet dosage form showed that the excipients not interfered in the estimation of drug. Hence, this method was specific and can be successfully used for the estimation of drug in bulk and in pharmaceutical dosage forms.

REFERENCES

1. Beckett A.H and Stenlake J.B. Text book of Pharmaceutical Chemisry.4 (2); 1998: 278-307.
2. Douglas Skoog A, James Hollar F and Timothy Nieman, Principles of Instrumental Analysis. Thomson Learning Inc., Singapore, 1998; 5:110, 300.
3. Sethi P.D, Quantitative Analysis of Drugs in Pharmaceutical Formulation, CBS Publishers and Distributors, 1997; 3:1-29, 50-64.
4. Mendham R.C, Denny J.D, Barnis M. and Thomas J.K, Vogel Text Book of Quantitative Chemical Analysis, 6thed. Pearson Education, 2003; 1, 676.
5. Sharma B.K. Instrumental methods of Chemical Analysis, 24th ed., Goel Publishing House, Mumbai, 2002; 2-149.
6. Chatwal G.R and Anand K.S. Instrumental methods of Chemical Analysis, 5thed Himalaya Publishing House, Mumbai, 2002; 2-149.
7. Robert D. Barun," Introduction to chemical analysis", Mc Grew- Hill Book co. Singapore, 1982.
8. Michael Swartz. HPLC Detectors, Journal of liquid chromatography.2010; 5: 2681-98.
9. Mitka M (May 2008). "Cholesterol drug controversy continues". JAMA 299 (19): 2266.
10. Taylor A.J., Villnes T.C., Stanek E.J., et al. (26 November 2009). "Extended-Release Niacin or Ezetimibe and Carotid Intima-Media Thickness". N Engl J Med 361 (22): 2113–223. http://guidance.nice.org.uk/TA132

11. Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP, et al. The target of Ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). Proc Natl Acad Sci U S A 2005; 102 (23):8132-7.
12. Temel Ryan E, Tang Weiqing Ma, Yinyan Rudel, Lawrence L, Willingham Mark C, Ioannou Yiannis A, Davies Joanna P, Nilsson Lisa-Mari Yu Liqing. Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of Ezetimibe J. Clin. Invest. 2007 0: JCI30060
13. DiPiro JT, Talbert RL, Yee GC, Marzke GR, Wells BG, Posey LM, editors. Pharmacotherapy: A Pathophysiologic approach. 7th Ed. New York: The McGraw-Hill Companies, Inc.; 2008.
14. Rossi S, Australian Medicines Handbook 2006. Adelaide: Australian Medicines Handbook; 2006. http://www.hc-sc.gc.ca/dhp-mps/medeff/advisories-avis/public/_2005/ezetrol_pa-apeng.php
15. "Simvastatin". The American Society of Health-System Pharmacists. Retrieved 3 April 2011.
16. "Gen-Simvastatin - Drug Factsheets - C-Health". Retrieved 2007-08-15.
17. R. Baselt, Disposition of Toxic Drugs and Chemicals in Man, 8th edition, Biomedical Publications, Foster City, CA, 2008, pp. 1431-1433.
18. Bays HE, Moore PB, Drehobl MA et al: Effectiveness and tolerability of simvastatin in patients with primary hypercholesterolemia: pooled analysis of two phase II studies. Clin Ther 2001, 23 (8), 1209-1230.
19. Jat .K, Chhipa RC, Sharma S., Spectrophotometric quantification of Carviedilol in bulk drug and tablets. Pharmacophore, 2010, 1(2), 90-95.
20. Jat R.K, Chhipa R.C and Sharma S, Spectrophotometric quantification of Etiricoxib in bulk drug and tablets using hydrotropic agents. Pharmacophore, 2010, 1(2), 96-102.
21. Jat R.K., Chhipa R.C. and Sharma S., quantitative estimation of clobazam in bulk drug and tablet .IJCPRR, 2011, 1(3), 18-24.
22. Jat R.K., Chhipa R.C. and Sharma S., Spectrophotometric Estimation of Fluvoxamine Maleate in Tablets Using Hydrotropic Agent, Int J Pharm Quality Res, 2011, 2(4); 73-75.
23. Lucie Novakova, Dalibor Satinsky, Petr Solich, HPLC methods for the determination of Simvastatin and Atorvastatin , Trends in Analytical Chemistry, 2008, 27(4), 352-367.
24. M. Lalitha Devi, K.B. Chandrasekhar, A validated stability-indicating RP-HPLC method for levofloxacin in the presence of degradation products, its process related

impurities and identification of oxidative degradants, journal of pharm and biomed Anal,2009 50(5), 710-717.

25. Indian Pharmacopeia 2007, Volume I, Published by The Indian Pharmacopoeia Commission, 477-478.
26. Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists. 1st ed. Published by Wiley-VCH; 2007; 369-382.
27. Stavros Kromidas. HPLC Made to Measure, A Practical Book for Optimization. Published by Wiley-VCH; 2006; 62-66.
28. Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists. 1st ed. Published by Wiley-VCH; 2007; 389-391.
29. Jenke D.R. Chromatographic method validation: A review of current practices and procedures. II. Guidelines for primary validation parameters. J. Liq. Chromatogr.19, 737–757, 1996.
30. Sharma Ajay, Sharma Rohit: Validation of analytical procedures: a comparison of ICH VS PHARMACOPOIEA (USP) and FDA. Inter National Research Journal of Pharmacy 2012; 3(6) 39-42.
31. Validation of analytical procedure: Methodology Q2B, ICH Harmonized Tripartite Guidelines, 1996:1-8.
32. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonized tripartite guideline Validation of analytical procedures: Text and Methodology Q2 (R1) 6 November 1996.
33. Ravichandran V, Shalini S, Sundram K. M and Harish Rajak: Validation of analytical methods – strategies & importance International Journal of Pharmacy and Pharmaceutical Sciences 2010; vol 2, suppl 3,
34. Tangri Pranshu, Rawat Prakash Singh, Jakhmola Vikash: Validation: A Critical Parameter for Quality Control of Pharmaceuticals. Journal of Drug Delivery & Therapeutics 2012; 2(3): 34-40.
35. ICH harmonized tripartite guideline. Impurities in New Drug products Q3B (R2) current step 4 versions dated 2 June 2006.
36. ICH, Q1A (R2), Stability Testing of New Drug Substances and Products, Geneva, Feb.2003.