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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANIOUS ESTIMATION OF EZETAMIBE AND ROSUVASTATIN IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

The HPLC method was developed by using, Zorbax SB C18 (150mmX 4.6mm id particle size 3.5µ) reverse phase packed with Octadecylsilane chemically bonded to porous silica or ceramic micro-particle with mobile phase 40:45:15 (v/v) potassium dihydrogen phosphate buffer (adjust the pH to 2.5 ±0.05 using dilute Ortho phosphoric acid), methanol and acetonitrile. Flow rate was 1.5ml / min with UV detection at 242 nm and the injection volume was set at 10 µl, with 10 min runtime. The developed method was validated by using various parameters according to ICH guidelines. It was validated for specificity, stability in analytical solution, linearity, precision, accuracy studies, LOD, LOQ, robustness and ruggedness. All the validation parameters were found to be well within the acceptance criteria. The system suitability parameters also reveals that the values within the specified limit for the proposed method. The theoretical plates for Ezetamibe and Rosuvastatin were found to be more than 2000 and the tailing factor is NMT 2.0.The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise. From the linearity studies, the specified range for Ezetamibe was found to be 25mcg to 75mcg and for Rosuvastatin was found to be 25mcg to 75mcg. It was evaluated by the visual inspection of the plot of Peak area vs. Concentration and the correlation was found to be linear. The accuracy was found that the recovery value of pure drug and sample is in between 99.94 % to 101.1% which indicates that the method is accurate. The system suitability should passes as per the test method at variable conditions, hence it was concluded that the test method was Robust.There is a wide scope for the development of new analytical methods for the assay of the above drugs. RP-HPLC technique has been used as a tool in the present work

KEY WORDS

Ezetamibe and Rosuvastatin, RP-HPLC

INTRODUCTION

Ezetamibe[1] is Anticholesteremic Agents, Cholesterol Absorption Inhibitors. Chemically Ezetamibe[1], 3R,4S)-1-(4-fluorophenyl) -3- [(3S)-3-(4-fluorophenyl) -3- hydroxypropyl] -4-(4-hydroxyphenyl)azetidin-2-one. Ezetimibe is primarily metabolized in the small intestine and liver via glucuronide conjugation (a phase II reaction) with subsequent biliary and renal excretion. In humans, ezetimibe is rapidly metabolized to ezetimibe-glucuronide.

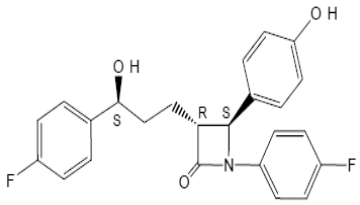


Figure 1: Chemical Structure of Ezetamibe

Rosuvastatin[2] is a Anticholesteremic Agents, HMG-CoA Reductase Inhibitors. Rosuvastatin[2], chemically it is found 3R, 5S, 6E) -7- [4- (4-fluorophenyl)-2-(N-methylmethanesulfonamido)- 6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid. Rosuvastatin is Rosuvastatin is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Rosuvastatin acts primarily in the liver. Decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL. Rosuvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL. In vitro and in vivo animal studies also demonstrate that rosuvastatin exerts vasculoprotective effects independent of its lipid-lowering properties. Rosuvastatin exerts an anti-inflammatory effect on rat mesenteric microvascular endothelium by attenuating leukocyte rolling, adherence and transmigration. The drug also modulates nitric oxide synthase (NOS) expression and reduces ischemic-reperfusion injuries in rat hearts. Rosuvastatin increases the bioavailability of nitric oxide by upregulating NOS and by increasing the stability of NOS through post-transcriptional polyadenylation. It is unclear as to how rosuvastatin brings about these effects though they may be due to decreased concentrations of mevalonic acid.

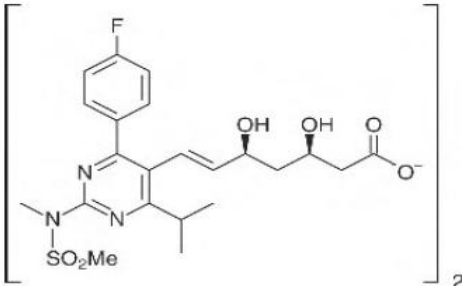


Figure 2: Chemical Structure of Rosuvastatin

The literature survey was carried out and it revealed that various methods have been reported in literature to detect and quantify the individual drugs. So there is a need to develop a simple, accurate and precise HPLC method for the simultaneous determination of the Ezetamibe and Rosuvastatin in combined tablet formulation.

The work of interest is Ezetamibe and Rosuvastatin in the combined dosage form. Validation of the method was done in accordance with ICH guidelines for the assay of active ingredients. Thus validated method can be recommended for the routine laboratory analysis.

MATERIALS & METHODS

Materials:

Analytically pure samples of *Ezetamibe and Rosuvastatin* were procured as gift samples from Dr. Reddy's Laboratories, (Hyderabad, India). Ezetamibe 10mg + Rosuvastatin 10mg -200mg tablets manufactured by NOVARTIS., USA were procured from a local pharmacy. The solvents for the experiment were selected based on the solubility test results of both the drugs. The solubility tests were performed using the common solvents like water, methanol (Merk), Acetonitrile (Merck). The analytical reagent grade Potassium dihydrogen ortho phosphate

(Merk) and orthophosphoric acid was used to prepare the mobile phase which is filtered through a nylon 0.45µm membrane filter paper[3-5].

CHROMATOGRAPHIC CONDITIONS:

The HPLC method was developed by using, Zorbax SB C18 (150mmX 4.6mm id particle size 3.5µ) reverse phase packed with Octadecylsilane chemically bonded to porous silica or ceramic micro-particle with mobile phase 40:45:15 (v/v) potassium dihydrogen phosphate buffer (adjust the pH to 2.5 ±0.05 using dilute Ortho phosphoric acid), methanol and acetonitrile. Flow rate was 1.5ml / min with UV detection at 242 nm and the injection volume was set at 10 µl, with 10 min runtime.

ASSAY OF FORMULATION:

PREPARATON OF STANDARD STOCK SOLUTION:

Accurately weighed quantity of 25 Ezetamibe mg of and 25 Rosuvastatin mg was transferred to a 100 ml volumetric flask, dissolved in 50 mL of methanol, sonicated for dissolve and the volume was made up to 100 mL with methanol.

PREPARATION OF SAMPLE SOLUTION:

Step 1

Weighed 20 tablets and determined the average weight and crushed to fine powder. Weighed accurately tablet powder equivalent to 50mg of Ezetamibe and Rosuvastatin transferred into 100 ml volumetric flask. Add methanol sonicated for 30 minutes in cold water, made up the volume with mobile phase. Mixed well and filtered through filtration unit.

Step 2

5 ml of above solution was diluted to 50 ml with methanol, so that the concentration of 50µg/ml respectively.

Step 3

Inject 10 µl of filtered portion of the sample preparation and Standard preparation into the chromatograph. Record the responses for the major peaks.

METHOD VALIDATION: The developed HPLC method for simultaneous determination of *Ezetamibe and Rosuvastatin* formulation was validated as per ICH guidelines.

Fig.No 3: Chromatogram of Blank for Assay

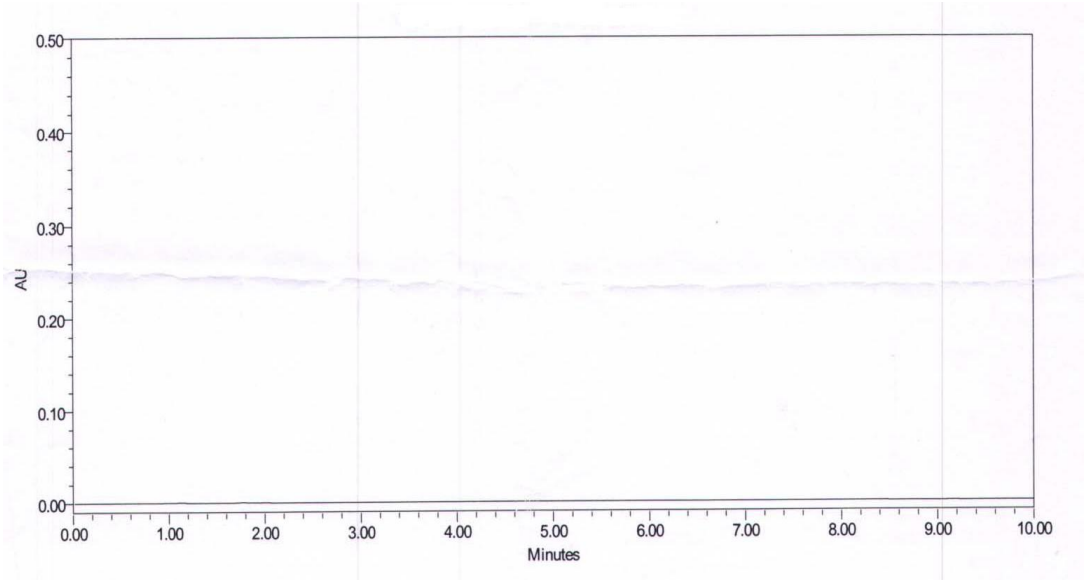


Fig.No 4: Chromatogram of Standard for Assay

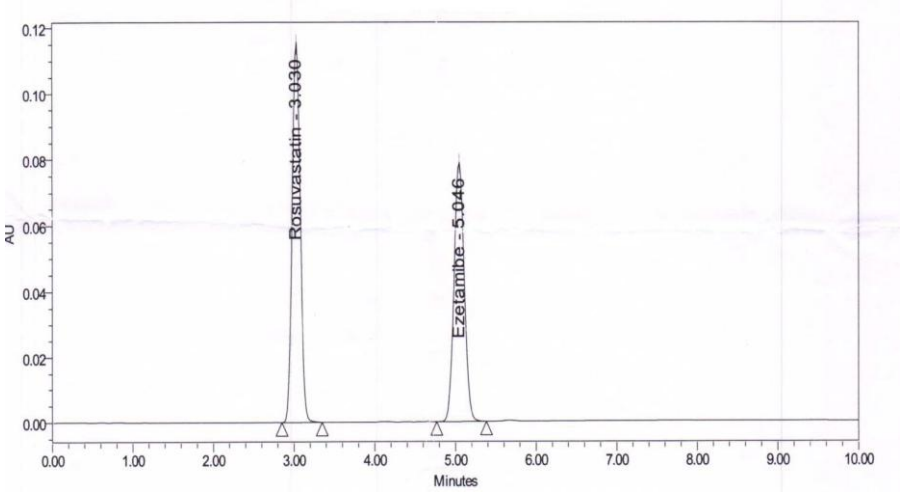


Fig. No 5: Chromatogram of Sample for Assay

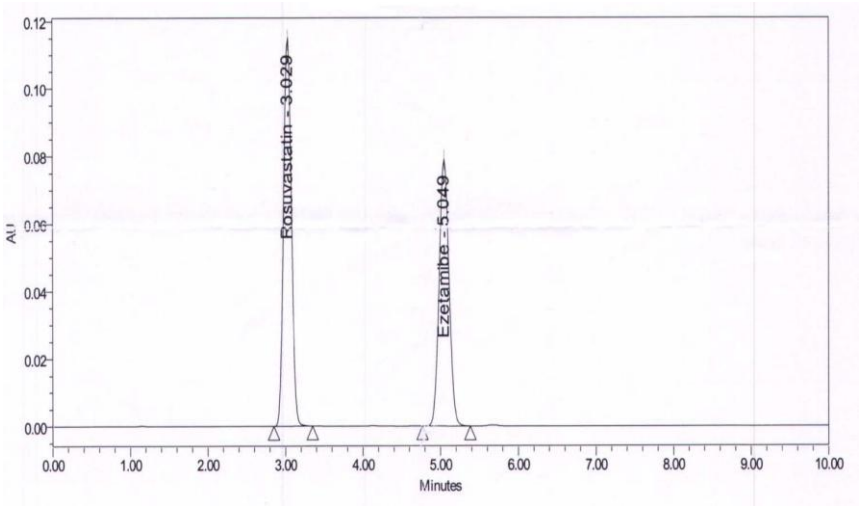


Table No1: Peak results of Standard & Test Chromatograms for Assay

Parameter	Standard		Sample	
	Rosuvastatin	Ezetamibe	Rosuvastatin	Ezetamibe
Retention time	3.030	5.046	3.029	5.049
Peak Area	812908	696745	811848	695649
USP Plate Count	2606	3756	4079	7448
Tailing Factor	1.3	1.2	1.0	1.0

The percentage assay is given by the following formula:

$$\% \, Assay = \frac{At}{As} \times \frac{Ws}{Ds} \times \frac{Dt}{Wt} \times \frac{P}{100} \times \frac{avg.weight}{label\ claim} \times 100$$

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 sample taken in mg.
Dt = sample dilution
Ds = standard dilution
P = Percentage purity of working standard

$$\text{Amount found (mg)} = \frac{\text{\% of Drug}}{100} \text{ x label claim}$$

The percentage purity of Ezetamibe and Rosuvastatin was found to be 99.84 % and 99.48% were within limit. From the results obtained it can be concluded that, this method is applied for routine analysis of simultaneous estimation of Ezetamibe and Rosuvastatin in their combined capsule dosage form.

Method validation was performed as per the ICH guidelines. The developed method was validated for the following parameters.

A. System suitability
B. Linearity
C. Specificity
D. Precision
E. Accuracy
F. LOD & LOQ
G. Robustness

SYSTEM SUITABILITY TEST (SST)

System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters were evaluated from tailing factor, retention times and theoretical plates of standard chromatograms.

LINEARITY:

The linearity of an analytical method was carried out to check 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. Different levels of standard solutions were Prepared and inject into the HPLC and the chromatograms were recorded.

Table No 2: Standard Dilutions for Linearity of Ezetamibe and Rosuvastatin

S.NO	Stock Solution of Ezetamibe and Rosuvastatin (ml)	Final Volume (ml)	Conc. Levels(µg/ml)
1	2.5	50	25
2	3.75	50	37.5
3	5	50	50
4	6.25	50	62.5
5	7.5	50	75

SPECIFICITY:

Specificity as the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the Chromatograms obtained from the drug standards with that of obtained from the tablet solution .The

retention times of the drug standards and the drug from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the capsules.

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements.

a) System Precision:

The system precision was carried out to ensure that the analytical system is working properly. Injected Standard preparation five times into the HPLC. Calculated the RSD for Ezetamibe and Rosuvastatin peaks in Standard preparation. The results obtained are tabulated. The retention time and area of five determinations is measured and % RSD should be calculated**.**

b) Method Precision:

In method precision, a homogenous sample of a single batch should be analyzed five times and was checked whether the method is giving consistent results for a single batch. The samples of Ezetamibe and Rosuvastatin were analysed five times. The % RSD was calculated for the sample.

ACCURACY:

The accuracy of an analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

The study was performed by making three different standard concentrations at50%, 100%, and 150% levels of known amounts of studied drugs. The accuracy of an analytical method should be established across its range. Finally, the final volume made up with solvent (methanol) and mixed well. The resulting mixtures were analysed by the proposed HPLC method at 242 nm. The excellent mean recoveries and standard deviation suggested good accuracy results of the propose method.

LIMIT OF DETECTION & QUANTIFICATION:[6,7]

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value and the quantitation 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 detection limit and the quantitation limit can be calculated based on the Standard Deviation of the Response and the Slope. .

The Detection Limit (DL) may be expressed as:

DL = 3.3F/S

The Quantitation Limit (QL) may be expressed as:

QL = 10F/S

Where,

F = Residual Standard deviation of the response,

S = Slope of the calibration curve.

Limit of Detection (LOD):

The parameter LOD was determined on the basis of response and slope of the regression equation.

The Detection Limit (DL) may be expressed as:

DL = 3.3F/S

Where,

F = Residual Standard deviation of the response,

S = Slope of the calibration curve.

The LOD for this method was found to be 0.718 µg/ml and 0.613 µg/ml for Ezetamibe and Rosuvastatin.

Limit of Quantification (LOQ):

The parameter LOQ was determined on the basis of response and slope of the regression equation.

The Quantitation Limit (QL) may be expressed as:

QL = 10F/S

Where,

F = Residual Standard deviation of the response,

S = Slope of the calibration curve.

ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and

provides an indication of its reliability during normal usage.

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and temperature which may differ but the responses were still within the specified limits of the assay. The standard solution, sample solution and sample solution spiked with impurities were injected into the chromatograph at varied conditions of flow ± 10%ml/min, mobile phase buffer pH ± 0.2 units and wavelength by + or -2nm.

a) Effect of variation of mobile phase buffer pH

A study was conducted to determine the effect of variation in mobile phase buffer pH by Changing the pH of mobile phase i.e. changes in buffer pH. Standard solution was prepared and injected into the HPLC system.

b) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping variation in flow rate + or – 10%. The effect of variation of flow rate was evaluated.

c) Effect of variation in wave length

A study was conducted to determine the effect of variation in wave length. Standard solution was prepared and injected into the HPLC system by keeping variation in wave length + or – 2 nm. The effect of variation of wave length was evaluated.

d) Effect of variation of Temperature

A study was conducted to determine the effect of variation in temperature. Standard solution was prepared and injected into the HPLC system by keeping variation in flow rate + or – 5^0C. The effect of variation of flow rate was evaluated.

RESULTS AND DISCUSSION

A new reversed phase high performance liquid chromatographic method has been developed and validated for the simultaneous estimation Ezetamibe and Rosuvastatin in tablet formulation. It is shown above that the method was accurate, reproducible, repeatable, linear, precise and selective, proving reliability of method. The run time is relatively short, i.e.10 min which enables rapid quantitation of many samples in routine quality control analysis of tablet formulation. The same solvent used throughout the experimental work and no interference from any excipients was observed.

These results show the method could find practical application as a quality control tool for the simultaneous estimation of the two drugs (Ezetamibe and Rosuvastatin) in their combined capsule dosage form (10/10 mg) in quality control laboratories.

The developed method was validated by using various parameters according to ICH guidelines. It was validated for specificity, stability in analytical solution, linearity, precision, accuracy studies, LOD, LOQ, robustness and ruggedness. All the validation parameters were found to be well within the acceptance criteria.

The system suitability parameters also reveals that the values within the specified limit for the proposed method. The theoretical plates for Ezetamibe and Rosuvastatin were found to be more than 2000 and the tailing factor is NMT 2.0.

The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise.

From the linearity studies, the specified range for Ezetamibe was found to be 25mcg to 75mcg and for Rosuvastatin was found to be 25mcg to 75mcg. It was evaluated by the visual inspection of the plot of Peak area vs. Concentration and the correlation was found to be linear.

The accuracy was found that the recovery value of pure drug and sample is in between 99.94 % to 101.1% which indicates that the method is accurate.

The LOQ for this method was found to be 2.176µg/ml and 1.858 µg/ml for Ezetamibe and Rosuvastatin.

The system suitability should passes as per the test method at variable conditions, hence it was concluded that the test method was Robust.

Table No 3: System Suitability

S.No.	System Suitability Parameters	Results	
		Rosuvastatin	Ezetamibe
1	Relative standard deviation(%RSD)		
2	Tailing factor(T_f)	1.0	1.0
3	Resolution (Rs)	-	9.37
4	Retention time(Rt)	3.030	5.046
5	Theoretical plates(N)	4069	7405

Table 4: Linearity of Rosuvastatin

S.NO	Concentration (µg/ml)	Peak Area
1	25	342372
2	37.5	521528
3	50	696745
4	62.5	870631
5	75	1042117
	Correlation coefficient(r^2)	0.999
	Slope (m)	13989
	Intercept	4758

Fig. No 6: Linearity Graph for Rosuvastatin

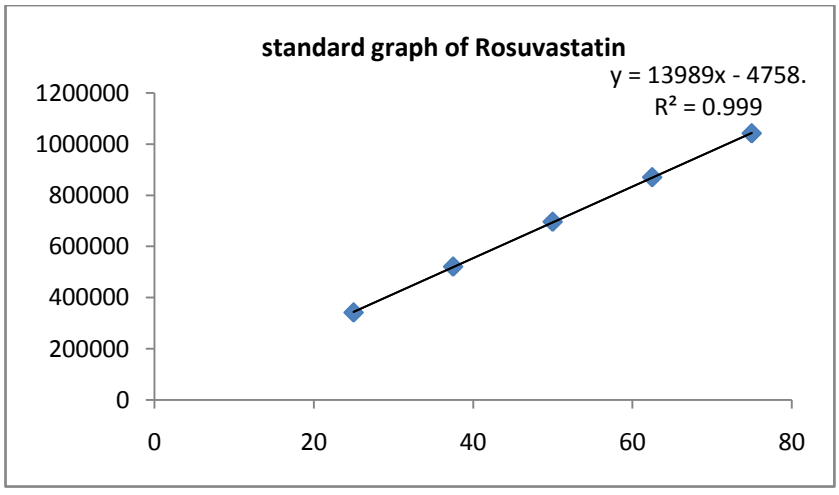


Table No 5: Linearity of Ezetamibe

S.NO	Concentration (µg/ml)	Peak Area
1	25	405484
2	37.5	601891
3	50	812908
4	62.5	1012135
5	75	1214632
Correlation coefficient(r^2)		0.999
Slope (m)		16228
Intercept		2006

Fig. No 7:Linearity Graph for Ezetamibe

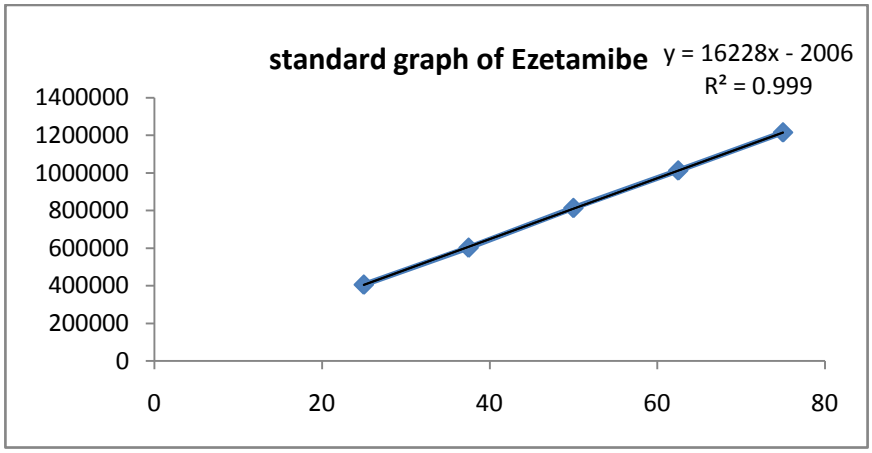


Fig. No 8:Chromatogram for Accuracy Level 50%

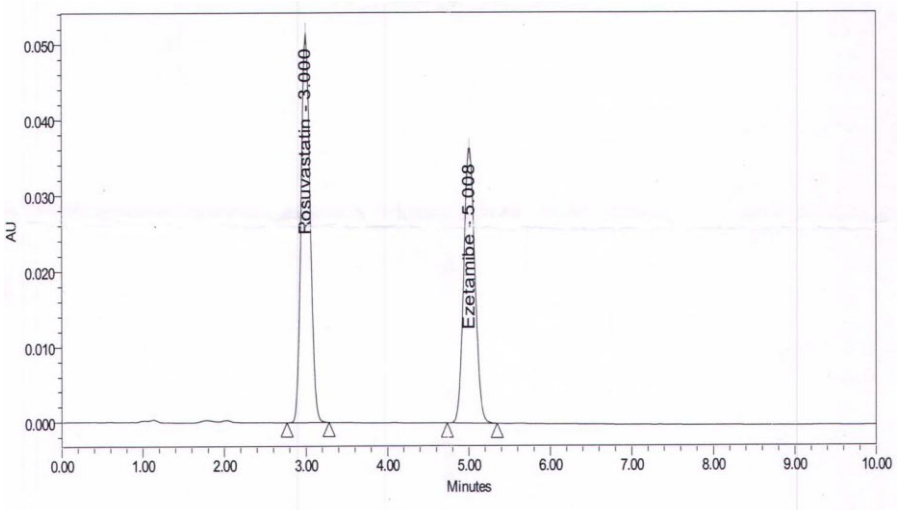


Fig. No 9:Chromatogram for Accuracy Level 100%

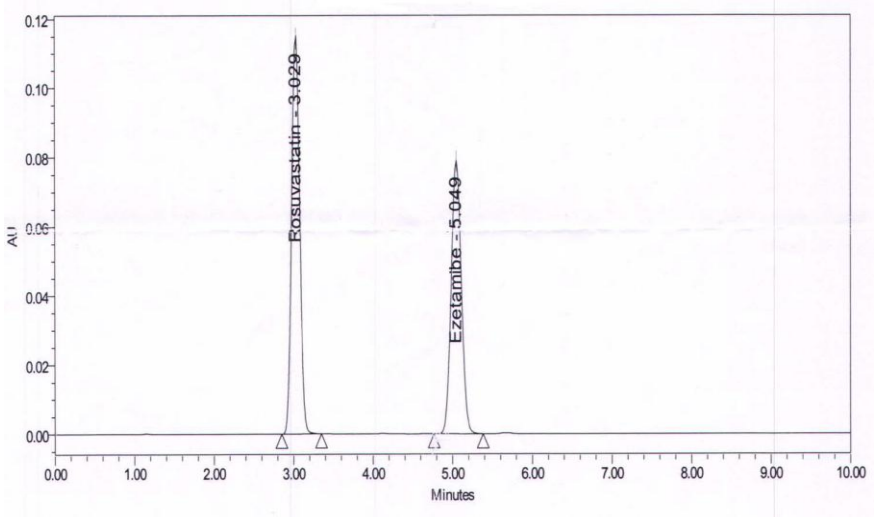
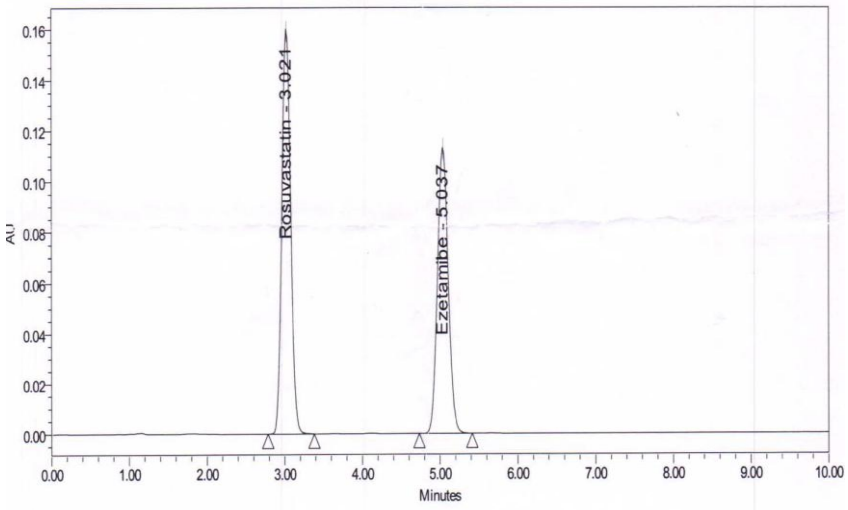


Fig. No 10: Chromatogram for Accuracy Level 150%



CONCLUSION

A simple, specific, precise, accurate, rapid and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Aliskiren and Amlodipine in tablet dosage form.

A simple, economic, accurate and precise HPLC method was successfully developed. The method was successfully validated in terms of linearity, precision, accuracy& robustness, LOD, LOQ as per ICH guidelines.

Using the optimized chromatographic conditions, chromatograms of Aliskiren and amlodipine were recorded. Calibration curves were obtained by using peak area vs. concentration. The accuracy studies were shown as % recovery for Aliskiren and Amlodipine at 50%, 100% and 150%. The limit of % recovered shown is in the range of 98-102% and the results obtained were found to be within the limits. Hence the method was found to be accurate. For Intra-Day & Inter-day precision studies of Aliskiren and Amlodipine was performed. %RSD was determined from the peak areas and was found to be not more than 2%. The proposed method is simple, accurate and rapid.

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. Limit of detection of 1.37741µg/ml & 4.17396µg/ml & limit of quantification of 0.73967µg/ml and 2.24142µg/ml for Aliskiren & Amlodipine respectively. For robustness studies the chromatograms were recorded for standard solutions of Aliskiren and

Amlodipine by changing flow rate. Robustness studies reveal that the method was reliable.

Hence the proposed method was found to be rapid, accurate, precise, robust and economical. The mobile phase is simple to prepare and economical. This method is also having an advantage of short retention time. The proposed method was a good approach for obtaining reliable results & found to be suitable for the routine analysis and quality control of pharmaceutical preparations containing these drugs either individually or in combination.

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REFERENCES

1. http://www.drugbank.ca/drugs/DB00973.
2. http://www.drugbank.ca/drugs/DB01098.
3. S. S. Dhaneshwar, S. R. Dhaneshwar, P. Deshpande, and M. Patil - Described a simple, accurate and precise RP-HPLC method for the Simultaneous densitometric estimation of Atorvastatin calcium And Ezetamibe as the bulk drug.. Journal of ACTA CHROMATOGRAPHIC, NO. 19, 2007
4. Anuradha K. Gajjar and Vishal D. Shah- Described a simple, accurate and precise RP-HPLC method for the Development and Validation of a Stability-Indicating Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Ezetimibe from Their Combination Dosage Forms. Eurasian Journal of Analytical Chemistry 5(3): 265-283, 2010
5. G.Krishnaveni , P.V.V. Sathyannarayana – Described a simple, accurate and precise RP-HPLC method for the Development and Validation of a Reversed-Phase HPLC Method for Simultaneous Estimation of Simvavastatin and Ezetimibe from Their Combination Dosage Forms. International Journal of Pharmaceutical and Life Sciences ISSN 2305-0330 Volume 2, Issue 2: March 2013
6. M. C. Sharma , Smita Sharmaa, D. V. Kohlib, A. D. Sharmac, Describes a First order Derivative A validated HPTLC method for determination of simultaneous estimation Rosuvastatin Calcium and Ezetimibe in pharmaceutical solid dosage form Archives of Applied Science Research, 2010, 2 (1) 1-7ISSN 0975-508X CODEN (USA) AASRC9
7. Baokar Shrikrishna B., Erande R.S. and Shaikh S.G., revealed a simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Ezetamibe. International Journal of Research in Pharmaceutical and Biomedical Sciences ISSN: 2229

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