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Development and Validation of RP-LC Method For Simultaneous Estimation of Rosuvastatin And Ezetimibe In Bulk and Its Pharmaceutical Formulations

Anil Shahaji Khile[1], N. Gayatri Devi[2], M. Subba Rao[2] and D. Ramachandran[2]*

1. Department of Chemistry, Rayalaseema University, Kurnool, Andhra Pradesh.

2. Department of Chemistry, Acharya Nagarjuna University, Nagarjuna nagar, Guntur. Andhra Pradesh, India.

ABSTRACT

A new simple, accurate, rapid and precise isocratic RP-HPLC was developed and validated for the determination of Rosuvastatin and Ezetimibe in Pharmaceutical tablet dosage form by droping method. The Method employs Shimadzu LC system on Hypersil ODS column (4.6 x 250 mm, 5 µm) and flow rate of 1.5ml/min with an injection volume 20µl. Buffer, Acetonitrile and Methanol was used as mobile phase in the composition of 40:30:30v/v. The Detection was carried out at 230nm. Linearity ranges for Rosuvastatin and Ezetimibe were 11-33µg/ml, 10-30µg/ml respectively for HPLC. Retention Time of Rosuvastatin and Ezetimibe were found to be 3.7 and 5.7 min respectively. Percent Recovery study values of Rosuvastatin and Ezetimibe were found 99.6-101.1% and 99.9-100.7% respectively. This newly developed method i.e. droping method was successfully utilized for the Quantitative estimation of Rosuvastatin and Ezetimibe in tablet dosage form. This method was validated for selectivity, accuracy, precision, and linearity, Ruggedness, Robustness and Stability Studies as per ICH guidelines.

Keywords: Liquid Chromatography; Rosuvastatin, Ezetimibe, Simultaneous estimation, Validation.

*Corresponding Author Email: dittakavirc@gmail.com

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INTRODUCTION

Rosuvastatin is a synthetic lipid lowering agent that blocks the production of cholesterol in the body, it is a competitive 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitor effective in lowering LDL cholesterol and triglycerides, developed for the treatment of dyslipidemia[1]. Chemically Rosuvastatin calcium is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-6-(1-methylehyl)-2-[methyl (methylsulphonylamino)]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid calcium[2] (Figure 1). Ezetimibe is a selective cholesterol absorption inhibitor, which potentially inhibits the intestinal absorption of cholesterol and related phytosterols by the small intestine without affecting absorption of triglycerides, fatty acids, bile acids and fat-soluble vitamins[3]. The drug is widely used in treatment of hypercholesterolemia and of sitosterolemia. Chemically ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone[4] (Figure 2).

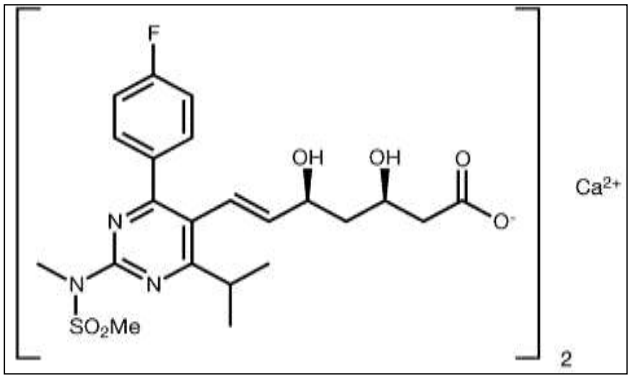

Figure 1: Chemical structure of Rosuvastatin Calcium

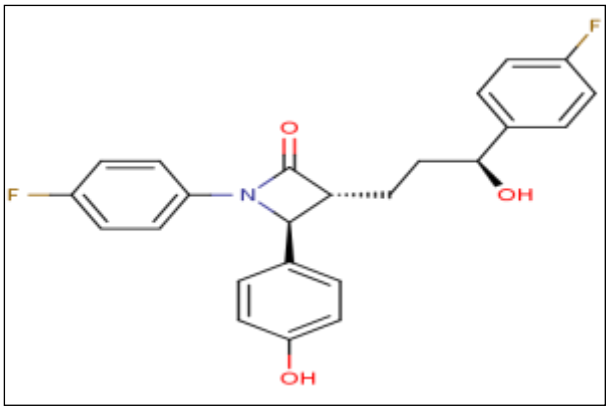

Figure 2: Chemical structure of Ezetimibe

Literature survey reveals that various spectrophotometric [5-8], HPLC [9-10], HPTLC [11], LC-MS [12-16] and capillary zone electrophoresis[17] methods have been reported for the determination of rosuvastatin in pure and pharmaceutical formulations and also various spectrophotometric [18-20], HPLC [21-24] and LC-MS [25-27] methods have been reported for the determination of ezetimibe in pure

and pharmaceutical formulations. Few analytical methods like spectrophotometric [28-29], spectrofluorometric[30], HPLC[31-33] and HPTLC[34-35] methods have been reported for the determination of rosuvastatin and ezetimibe in combined dosage form. So an attempt was made to report a simple, rapid, sensitive, accurate and precise HPLC method for the determination of rosuvastatin and ezetimibe in combined tablet dosage form.

MATERIALS AND METHOD

Chemicals and Reagents

Analytical-grade Ammonium acetate, Glacial acetic acid, Methanol, Acetonitrile and Water HPLC-grade, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, and India. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation and Chromatographic Conditions

Instrumentation

Shimadzu PDA detector Separation Module, equipped with LC 2010 CHT, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

Mobile phase preparation

A mixture of 40 volumes of 0.2% acetic acid in water, 30 volumes of Acetonitrile and 30 volumes of Methanol, filter and degas.

Diluent preparation

A mixture of 50 volumes of buffer solution (Prepared by dissolving 0.77g of ammonium acetate in 1000ml of water) 50 volumes of acetonitrile.

Standard preparation:

Weigh accurately 22mg of Rosuvastatin Calcium and 20mg of Ezetimibe in 100ml volumetric flask and dissolve in diluent and sonicate for 10minutes and make up to the volume.

Further diluted 5ml of above solution was transfer in to 50ml volumetric flask and diluted with diluent. Centrifuge the solution in 3000 rpm for 5minutes and inject into the chromatogram.

Sample preparation:

Weigh and transfer 10 whole tablets in a 500ml volumetric flask add 200ml diluent and sonicate for 20minutes and cool, after cooing make up to the volume. Further dilute 10ml of this solution to 100ml with diluent. Centrifuge the solution in 3000 rpm for 5minutes and inject into the chromatogram.

Chromatographic conditions

Hypersil ODS column (250 x 4.6 mm, 5µ) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.5mL/min. The sample injection volume was 20 µL. The photodiode array detector was set to a wavelength of 230 nm for the detection and Chromatographic runtime was 10 minutes.

RESULTS AND DISCUSSION

Method development

To develop a suitable and robust LC method for the determination of Rosuvastatin Calcium and Ezetimibe, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil ODS 3 Column (250×4.6mm, 5µm). Mobile phase was used Buffer (0.2% Acetic Acid): Acetonitrile (55:45 v/v).Detector wavelength 230nm, column temperature ambient, Injection volume 20 µL and Flow rate 1.0 ml/min used. Peak shapes were not satisfactory more retention time was observed for both Rosuvastatin Calcium and Ezetimibe and the retention time of Rosuvastatin Calcium and Ezetimibe were found to be 8.25 and 16.55 min respectively.

For next trial the Column was changed Hypersil ODS column (250×4.6mm, 5µm) Column was used. The mobile phase composition was Buffer (0.2% Acetic Acid): Acetonitrile (55:45 v/v) Filtered through 0.22 µ membrane filter and degassed. Detector wavelength 230 nm, column temperature ambient, Injection volume 20 µL and Flow rate 1.5 ml/min used. Run time 10minutes. More retention time for Ezetimibe was observed and also the plate count was not within the limits.

For next trial mobile phase composition was changed Buffer (0.2% Acetic Acid): Acetonitrile: Methanol (40:30:30 v/v/v). Filtered through 0.22 µ membrane filter and degassed. Hypersil ODS column (250×4.6mm, 5µm) Column was used. Detector wavelength 230 nm, column temperature ambient, Injection volume 20 µL and Flow rate 1.5 ml/min used. Run time 10minutes.

Peak shape was satisfactory in both standard and sample preparations. Retention time of Rosuvastatin Calcium and Ezetimibe were found to be 3.7 minutes and 5.7 minutes. The chromatogram of Rosuvastatin Calcium and Ezetimibe standard using the proposed method is shown in (Figure 3.) System suitability results of the method are presented in **Table 1.**

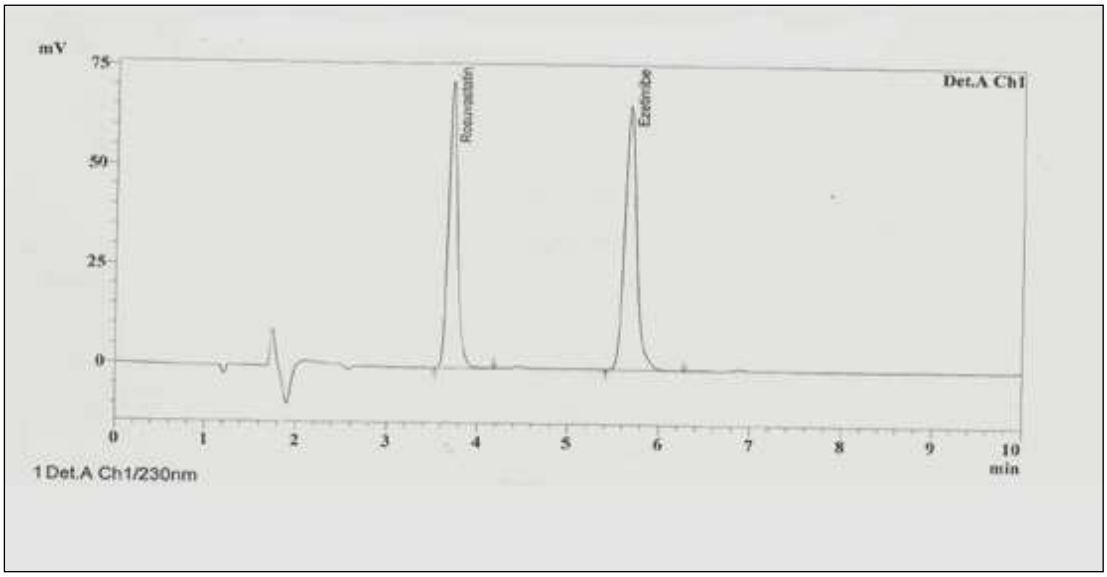

Figure 3: A typical HPLC Chromatogram showing the peak of RSC and EZM

Method validation

The developed RP-LC method extensively validated for assay of Rosuvastatin Calcium and Ezetimibe using the following Parameters.

Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Fig. 1.4)** showed no peaks at the retention time of Rosuvastatin Calcium and Ezetimibe peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Rosuvastatin Calcium and Ezetimibe in tablets. Similarly Chromatogram of Placebo solution **(Fig. 1.5)** showed no peaks at the retention time of Rosuvastatin Calcium and Ezetimibe peak. This indicates that the Placebo used in sample preparation does not interfere in estimation of Rosuvastatin Calcium and Ezetimibe in Rosuvastatin Calcium and Ezetimibe tablets. The chromatogram of Rosuvastatin Calcium and Ezetimibe.

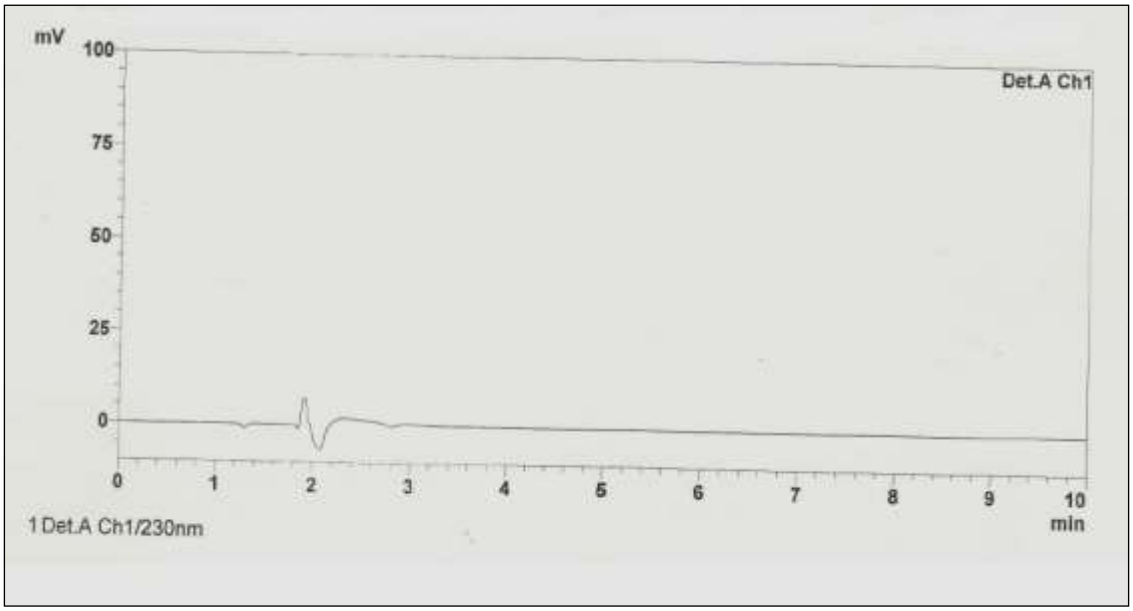

Figure 1.4: HPLC Chromatogram showing the no interference of Blank for RSC and EZM

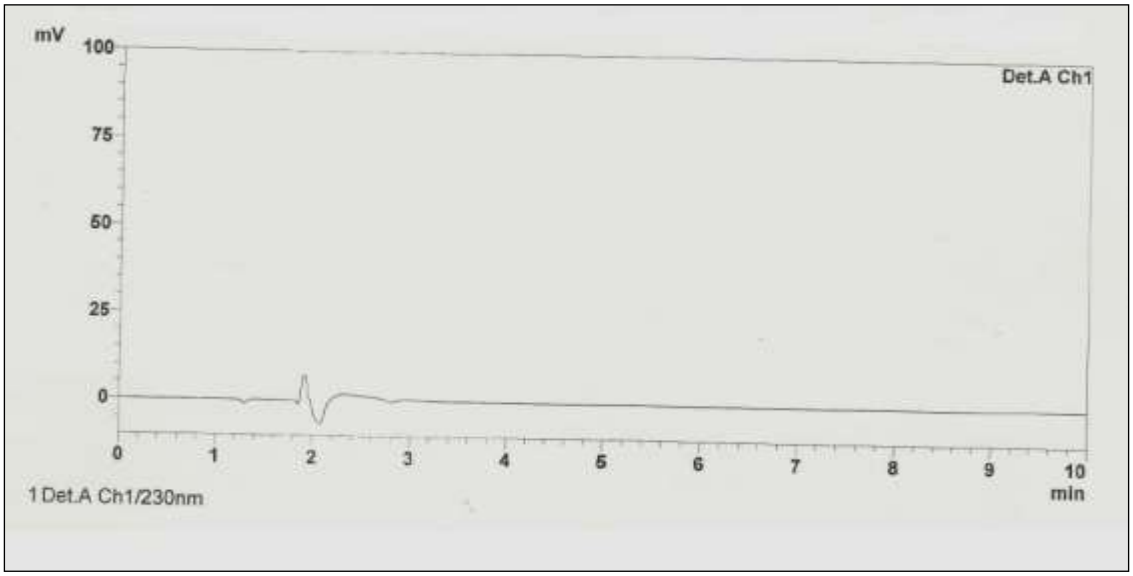

Figure 1.5: HPLC Chromatogram showing the no interference of placebo for RSC and EZM

Table 1.1: System suitability parameters for RSC and EZM by proposed method

Parameters	Rosuvastatin Calcium	Ezetimibe
Retention time (min)	3.7	5.7
No. of Theoretical plates	5751	8547
Tailing factor	1.3	1.3

Precision

The method precision study for six sample preparations in marketed samples showed a RSD of 0.09% for Rosuvastatin Calcium. Similarly the method precision study for six sample preparations in marketed samples showed a RSD of 0.05% for Ezetimibe.

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 1.26 % of Rosuvastatin Calcium and 1.39% Ezetimibe. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Table 2: Method Precision studies for RSC and EZM by proposed method

S.No	RT of Rosuvastatin	Peak area of Rosuvastatin	RT of Ezetimibe	Peak area of Ezetimibe
Injection-1	3.7	509277	5.6	612698
Injection-2	3.7	508935	5.6	612417
Injection-3	3.7	508418	5.6	612165
Injection-4	3.7	509690	5.6	612762
Injection-5	3.7	509399	5.6	612989
Injection-6	3.7	509249	5.6	612923
Mean	3.7	509161	5.6	612659
SD	0.00	438	0.00	314
% RSD	0.00	0.09	0.00	0.05

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Rosuvastatin Calcium and Ezetimibe, analyzed as per the proposed method. The amount of the each drug present, percentage recovery, percentage relative standard deviation (% RSD) was calculated. 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. The accuracy studies showed % recovery of the Rosuvastatin and Ezetimibe in the range 99.6-101.1%, 99.9-100.7%. From the data obtained which given in **Table 3** the method was found to be accurate.

Table 3: Recovery studies for Rosuvastatin Calcium and Ezetimibe by proposed method

S. No	Accuracy %	Amount present (mg)		Amount recovered (mg)		% Recovery	
		Rosu	Eze	Rosu	Eze	Rosu	Eze
1.	80%	89.9	80.0	90.4	80.3	100.5	100.4
2.		89.4	80.3	90.4	80.3	101.1	100.1
3.		90.1	79.8	90.1	80.3	100.3	100.7
1.	100%	110.2	100.1	110.9	100.1	100.6	100.0
2.		110.6	100.2	110.9	100.1	100.3	99.9
3.		110.4	100.2	110.9	100.1	100.4	99.9
1.	120%	135.6	120.4	136.3	120.6	100.5	100.2
2.		135.5	120.6	136.4	120.6	100.6	100.2
3.		136.9	120.6	136.4	120.6	99.6	100.4

Linearity of detector response

The standard curve was obtained in the concentration range of 11-33 µg/ml for Rosuvastatin Calcium and 10-30 µg/ml for Ezetimibe. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Figure 6** For Rosuvastatin Calcium and **Figure 7** For Ezetimibe to demonstrate the linearity of the proposed method. From the data obtained which given in **Table 4** For Rosuvastatin Calcium and Ezetimibe the method was found to be linear within the proposed range.

Table 4: Analytical performance parameters for linearity

S.No	Concentration	Rosuvastatin		Ezetimibe	
		Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1.	50%	11	584086	10	353403
2.	60%	13.2	706797	12	425820
3.	80%	17.6	925879	16	559878
4.	100%	22	1168961	20	706019
5.	120%	26.4	1399447	24	845025
6.	150%	33	1754112	30	1061006
Linearity range (µg/ml)		11-33		10-30	
Y Intercept		26.21		800.2	
Slope		11677		7064	
Correlation co-efficient (r^2)		0.999		0.999	

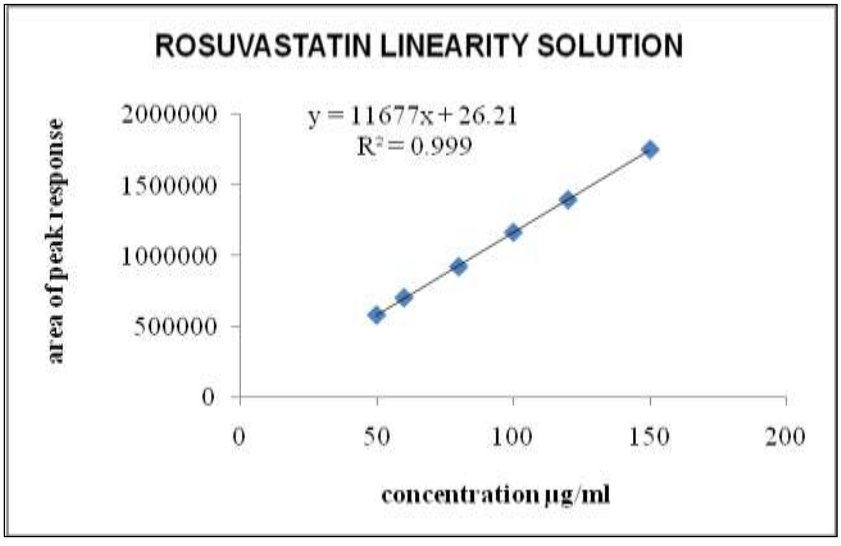

Figure 6: Calibration curve for Rosuvastatin Calcium

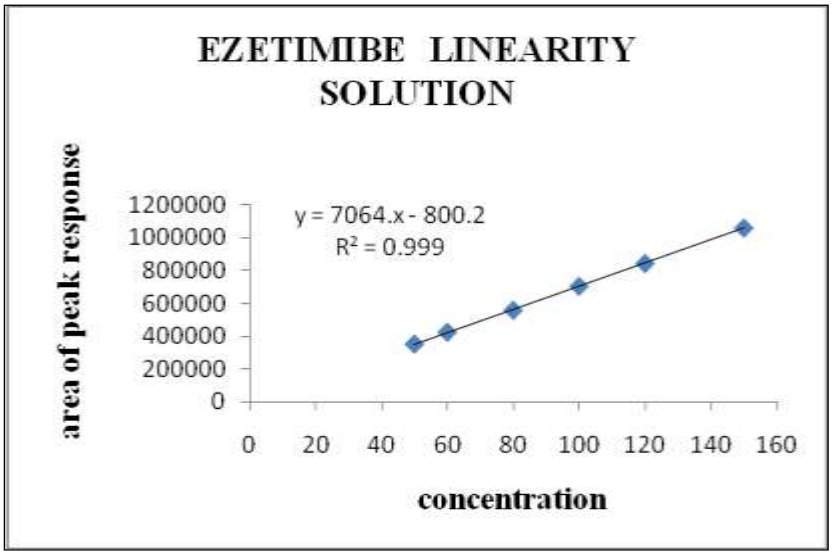

Figure 7: Calibration curve for Ezetimibe

CONCLUSION

An RP-HPLC method for simultaneous estimation of Rosuvastatin Calcium and Ezetimibe was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 11-33µg/ml for Rosuvastatin Calcium and 10-30µg/mL for Ezetimibe. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed. We have developed a fast, simple and reliable analytical method for determination of Rosuvastatin Calcium and Ezetimibe in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Rosuvastatin Calcium and Ezetimibe. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Rosuvastatin Calcium and Ezetimibe in bulk, its different pharmaceutical dosage forms.

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