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Simultaneous Quantification of Rosuvastatin Calcium and Ezetimibe in Combined Tablet Dosage Form Using HPTLC Method

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Rahul Rajan R.S [1]*, Anitha Thomas[2]

1, Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram, Kerala, India.695011.*

2, Assistant Professor, Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram., Kerala, India.695011.

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ABSTRACT

The present work describes the development and validation of a new simple, accurate, precise, and selective high-performance thin layer chromatographic (HPTLC) method for the determination of rosuvastatin calcium (ROS) and ezetimibe (EZT) in combined tablet dosage form. The stationary phase consists of Silica Gel G 60 F254 and the mobile phase consists of dichloromethane: ethyl acetate: glacial acetic acid (8:1.5:0.5v/v/v) and detection was carried out at 245 nm. The R_f values of ROS and EZT were found to be 0.23 ±0.02 and 0.71 ±0.02 respectively. Validation of the developed method was carried out as per ICH guidelines Q2(R2).A good linear relationship was found with the calibration curve for linearity in the concentration range of 1000-1500ng/spot and 500-2500ng/spot for ROS and EZT with R^2 values of 0.9993 and 0.9983 respectively. For ROS and EZT the LOD was found to be 293.7ng/spot and 128.16ng/spot respectively. The LOQ value for ROS and EZT was found to be 890.10 ng/spot and 388.38 ng/spot respectively. % recovery for ROS was found to be 103.06% to 107.99% and for EZT was found to be 101.40% to 105.48%. The developed method can be used for the simultaneous quantification of these drugs in combined tablet dosage form as well as for routine analysis in quality control laboratories.

INTRODUCTION

Method development and validation are critical components of drug development as well as chemical manufacture and controls (CMC). The aim of method development and validation is to guarantee the accuracy, precision, and dependability of the techniques used to test the identification, potency, purity, and stability of pharmaceuticals. The quality, safety, and effectiveness of pharmaceutical products are crucially monitored during the product's development process with the use of analytical procedures.[1]

Hypolipidemic medication is any substance that lowers blood levels of lipids, lipoproteins, or lipid-protein complexes. Cholesterol is bound by lipoproteins, which can build up in blood vessels. Excessive concentrations of some lipoproteins, specifically low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), have been linked to an increased risk of heart attack, stroke, and coronary artery disease. Statins are hypolipidemic medications that inhibit HMG-CoA (5-hydroxy-3-methylglutaryl-coenzyme A) reductase, an enzyme necessary for cholesterol production. Statins include Rosuvastatin, lovastatin, pravastatin, and simvastatin, as examples. Although statins are usually very safe, weariness and muscle soreness are possible adverse effects.[2]

Rosuvastatin Calcium is the calcium salt of rosuvastatin, a statin with antilipidemic properties. Rosuvastatin preferentially and competitively binds to and inhibits hepatic hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase, an enzyme that converts HMG-CoA to mevalonate, a precursor to cholesterol. This decreases hepatic cholesterol levels while increasing LDL cholesterol absorption.[3]

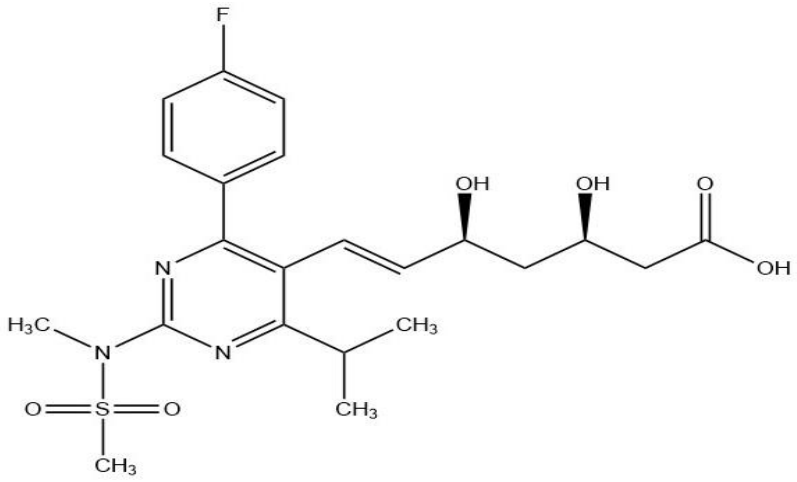

Fig 1: Structure of Rosuvastatin Calcium

Ezetimibe is a beta-lactam that is azetidine-2-one modified at 1, 3, and 4 with p-fluorophenyl, 3-(p-fluorophenyl)-3-hydroxypropyl, and 4-hydroxyphenyl groups, respectively (the 3R,3'S,4S enantiomers). It functions as an anticholesteremic, antilipidemic, and antimetabolite. It is classified as azetidine, an organofluorine molecule, and a beta-lactam.[4]

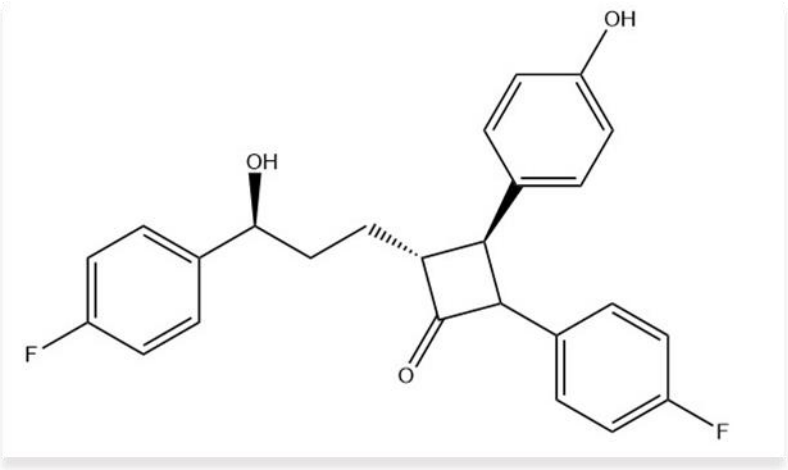

Fig 2: Structure of Ezetimibe

A combination of ROS (Rosuvastatin Calcium) and EZT (Ezetimibe) can reduce low-density lipoprotein (LDL) cholesterol levels by about 70% in high-risk hypercholesterolemic patients. The ROS-EZE combo dramatically lowers LDL cholesterol levels compared to ROS alone.[5]

Various analytical methods, including UV spectrophotometry,[6-9] High-Performance Liquid Chromatography (HPLC),[10-15] and High-Performance Thin Layer Chromatography (HPTLC), [16-18] have been used to detect rosuvastatin calcium and ezetimibe in pharmaceutical formulations, either alone or in combination with other drugs. The current work aims to develop and validate an HPTLC method in accordance with ICH guidelines Q2(R2) for the simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in combined tablet dosage form.

2.0 MATERIALS AND METHODS

2.1 Reagents and Chemicals:

Reference standards of Rosuvastatin Calcium were brought from Relimark Products and Services, Hyderabad, and Ezetimibe were brought from Yarrow Chem Products, Mumbai. The tablet dosage form used in this study is Rozavel EZ labeled to contain Rosuvastatin

Calcium 20 mg and Ezetimibe 10 mg by SUN Pharma were procured from the local market. Methanol and Dichloromethane HP LC grade procured from Merck Specialities (P) Ltd. Glacial acetic acid, procured from Merck Life Sciences (P) Ltd. Ethyl acetate, procured from Kanton Laboratories (P) Ltd.

2.2 Instrumentation and chromatographic conditions:

Chromatographic separation of drugs was performed on Merck Precoated Silica gel G 60 F 254 Aluminium plate (20 × 10 cm with 250 µm thickness) using a CAMAG Linomat 5 sample applicator. The development of plate was carried as ascending mode on a 20 × 10 cm CAMAG Twin Trough chamber using dichloromethane: ethyl acetate: glacial acetic acid (8:1.5:0.5v/v/v) as mobile phase. The sample was applied as a band with a 7mm width using a 100µL syringe (Hamilton, Switzerland). The mobile phase was saturated for 20 minutes at room temperature. The developed plate was scanned and visualized using CAMAG Scanner 4 with VISIONCATS software version 3.0 and CAMAG TLC VISUALIZER 2.

2.3 Preparation of standard drug solutions

Weighed accurately 10 mg and 5mg of Rosuvastatin Calcium RS and Ezetimibe R S and transferred to a 10 ml standard flask. It is dissolved in a sufficient quantity of HPLC-grade methanol, and the volume was made up to the mark using the same. The solution had a concentration of 1000 µg/ml of Rosuvastatin Calcium and 500 µg/ml of ezetimibe.

2.4 Selection of detection wavelength

After chromatographic development, bands were scanned in the region of 200-400 nm. It was observed that both drugs showed considerable absorbance at 254 nm. So, 254 nm was selected as the wavelength for detection.

2.5 Development of the solvent system

The selection of the mobile phase depends on the polarity of the analyte (Rosuvastatin Calcium and Ezetimibe) and the adsorption properties of the stationary phase. The solubility of drugs also plays a major role in the selection of suitable solvent systems. The solvent system was selected through a series of trial-and-error methods.

2.6 Preparation of Stock solution of standard drug mixture

Weighed accurately 10 mg of Rosuvastatin Calcium and 5 mg of Ezetimibe separately and transferred to a 10 ml standard flask. The drug mixture was dissolved in a sufficient quantity of HPLC-grade methanol, and the volume was made up to the mark using the methanol to obtain a concentration of 1000 µg/ml Rosuvastatin Calcium and 500 µg/ml Ezetimibe. From this 3 µL was spotted to obtain a concentration of 3000 µg/ml Rosuvastatin Calcium and 1500 µg/ml Ezetimibe.

2.7 Preparation of calibration curve of Rosuvastatin Calcium and Ezetimibe HCl and analysis of combined dosage form

a) Preparation of standard and sample solutions:

Standard solution of Rosuvastatin Calcium RS:

- Weighed accurately 10 mg of Rosuvastatin Calcium RS and transferred it to a 10 ml standard flask. It is dissolved in a sufficient quantity of HPLC-grade methanol, and the volume was made up to the mark using the same. The solution had a concentration of 1000 µg/ml of Rosuvastatin.
- From this 1µL, 2µL, 3µL, 4µL, and 5µL were spotted in five different bands to obtain the concentration of 1,2,3,4 and 5 µg/band.

Standard solution of Ezetimibe HCl RS:

- Weighed accurately 5 mg of Ezetimibe RS and transferred it to a 10 ml standard flask. It is dissolved in a sufficient quantity of HPLC-grade methanol, and the volume was made up to the mark using the same. The solution had a concentration of 500 µg/ml of Ezetimibe.
- From this 1µL, 2µL, 3µL, 4µL, and 5µL were spotted in five different bands to obtain the concentration of 0.5,1,1.5, 2, and 2.5µg/band.

2.8 Preparation of sample solution

Twenty tablets of ROSAVEL EZ were weighed and finely powdered with the help of a mortar and pestle. The average weight of a tablet was determined. A quantity of powder equivalent to 10 mg of Rosuvastatin Calcium and 5 mg of Ezetimibe HCl was accurately weighed, transferred to a stoppered flask, and extracted with 5 ml methanol initially by sonication for

10 minutes. Then the solution was filtered through Whatman No.1 filter paper to a 10 ml standard flask. The residue was extracted twice with 2 ml methanol HPLC grade and transferred to the same standard flask through the same filter paper. The volume was finally made up to 10 ml with methanol. The resulting solution had concentration of a 1000 µg/ml of Rosuvastatin calcium and 500 µg/ml of Ezetimibe HCl. From this solution, 3µL was spotted for the estimation of Rosuvastatin calcium and Ezetimibe HCl in the tablet dosage form concentration of 3000 µg/ml of Rosuvastatin calcium and 1500 µg/ml of Ezetimibe HCl.

2.9 Scanning and Integration of Chromatogram

The developed plate was mounted on the CAMAG HPTLC Scanner 4 operated by VISIONCATS software version 3.0 The slit width for scanning was 6.00 × 0.45 mm. and the scanning speed was 20 mm/sec. The chromatogram of Rosuvastatin calcium and Ezetimibe results are furnished in Fig 5a and 5b. The calibration curve graph of concentration v/s peak area of Rosuvastatin calcium and Ezetimibe is shown in Fig 8a and 8b. The developed plate is shown in the figure 3. The Chromatogram of the standard mixture and sample are shown in Fig 9a and 9b.

2.10 Validation of Proposed Method

Validation of the proposed method was done as per ICH Q2(R2) guidelines.

2.10.1 Accuracy

Accuracy of the proposed method was determined by the recovery study. The recovery studies were performed by standard addition method at the three concentrations, (80%, 100%, 120%), and percentage recovery was calculated. The results are shown in Tables 3a, 3b, and 3c.

2.10.2 Precision

Precision was determined at two levels: Repeatability and intermediate precision.

The repeatability study of the method was done by using 100% test concentration. For this, a chromatogram of the solution containing 3000 µg/ml Rosuvastatin Calcium and 1500 µg/ml Ezetimibe HCl was developed. The peak area was scanned six times at 254 nm and data is shown in the table 4a and 4b. The statistical validation data is shown in Table 4c.

In intermediate precision, the inter-day precision was done by scanning the chromatogram of three concentrations of both drugs three times on three days. Standard solutions of both Rosuvastatin Calcium and Ezetimibe HCl having concentration 1000 µg/ml were prepared. From this solution, 3µL is spotted as band to get 3000 ng/band. Later the chromatograms were developed and scanned at 254 nm. The peak area was measured three times for each band on three consecutive days. The statistical validation data is shown in Table 5c.

2.10.3 Linearity and Range

The linearity study was conducted to evaluate the linear relationship across the range of analytical procedures. Linearity was determined by using five different concentrations of each drug. Chromatogram was developed at 254 nm and peak height and peak were determined. The calibration graph (Concentration v/s Peak area) was plotted for each drug and from this linearity was determined for each drug. The data showing the linearity of the developed method is furnished in Table 6.

2.10.4 LOD and LOQ

The LOD and LOQ were estimated from five calibration curves drawn for each drug in their respective linearity range and calculated by the equation. The results are shown in Table 7.

3.0 RESULTS AND DISCUSSION

Method Development

Rosuvastatin Calcium and Ezetimibe were soluble in methanol; therefore, methanol was selected as the solvent. A solvent system consisting of dichloromethane: ethyl acetate: and glacial acetic acid (8:1.5:0.5v/v/v) gives dense and compact bands with appropriate Rf values. Hence it was selected as the mobile phase for the quantification of Rosuvastatin Calcium and Ezetimibe in combined tablet dosage form. The present HPTLC method for quantifying Rosuvastatin Calcium and Ezetimibe in combined tablet dosage form was revealed as simple, accurate, and precise with Rf values shown in Table 1**.**

3.1 Determination Rf value

Table 1: Rf values of the drugs

Drug	Rf values
Rosuvastatin calcium	0.231
Ezetimibe	0.715

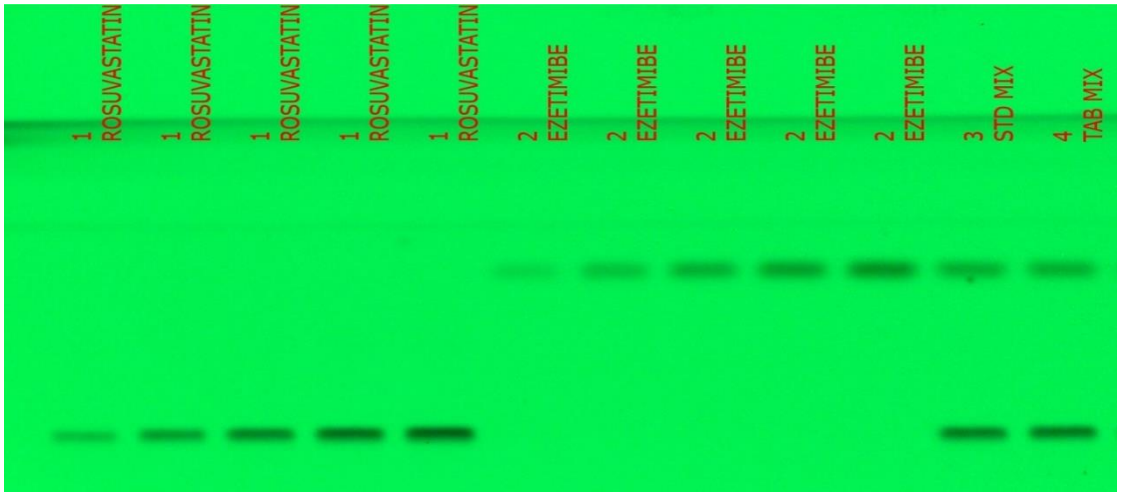

Fig 3.: Photograph of Developed HPTLC Plate in Short-Wave UV Light (254nm)

The figure 3 shows the photograph of the developed plate in UV light of 254nm. The first 5 bands represent the band of Rosuvastatin Calcium in a concentration range from 1000 – 5000 ng/band. The next 5 bands represent Ezetimibe in a concentration range from 500 – 1500 ng/band. The last 2 bands represent the standard drug mixture and tablet mixture in a concentration of 3000 ng/band for Rosuvastatin Calcium and 1500 ng/band for Ezetimibe.

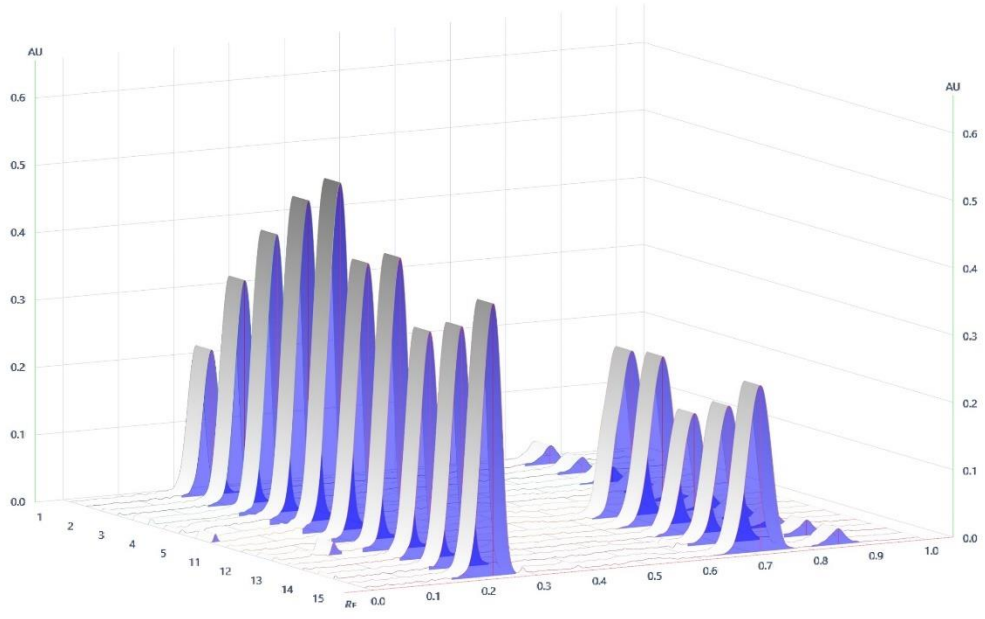

Fig 4: Overall 3D diagram for ROS and EZT

The figure 4 represents a 3D diagram of Rosuvastatin Calcium and Ezetimibe in a concentration range from 1000 – 5000 ng/band for ROS and 500 – 1500 ng/band for EZT.

3.2 Chromatogram of drugs with Rf value.

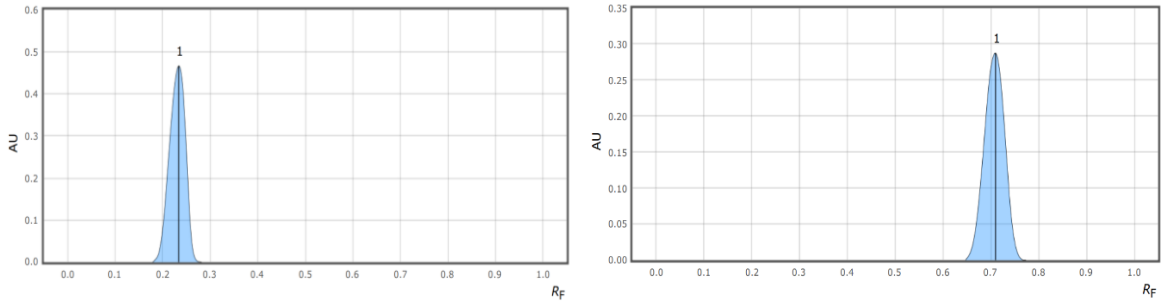

Fig 5a: Chromatogram of Rosuvastatin calcium with Rf 0.231 Fig 5b: Chromatogram of Ezetimibe with Rf 0.715

The figure 5a and 5b represent the developed chromatogram of ROS and EZT with their Rf values.

3.3 Chromatogram of Rosuvastatin Calcium

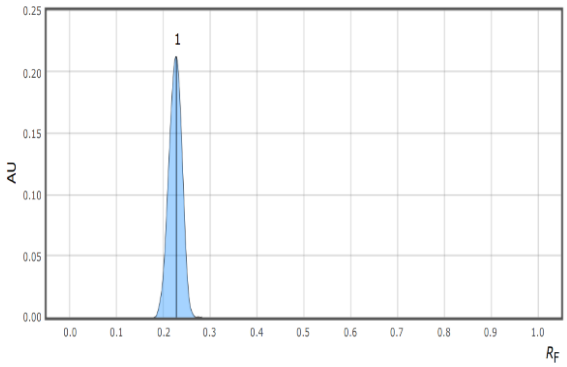

Fig 6a: Rosuvastatin calcium 1000 ng/band

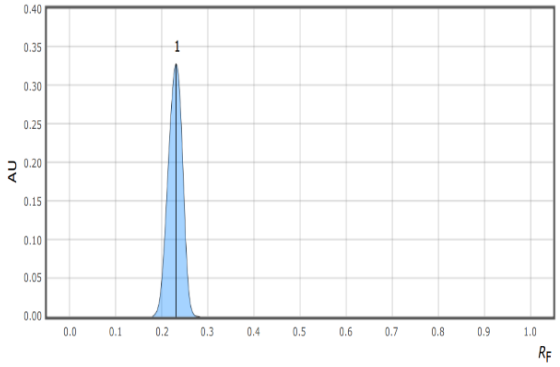

Fig 6b: Rosuvastatin calcium 2000 ng/ band

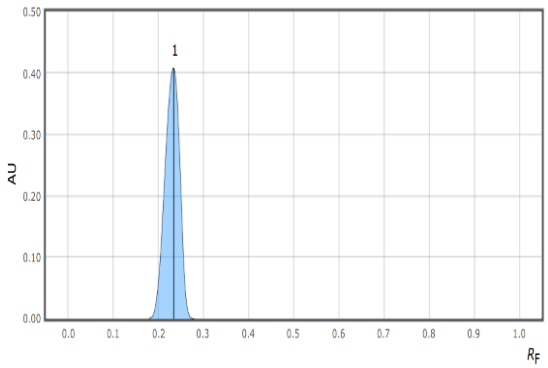

Fig 6c: Rosuvastatin calcium 3000 ng/ band

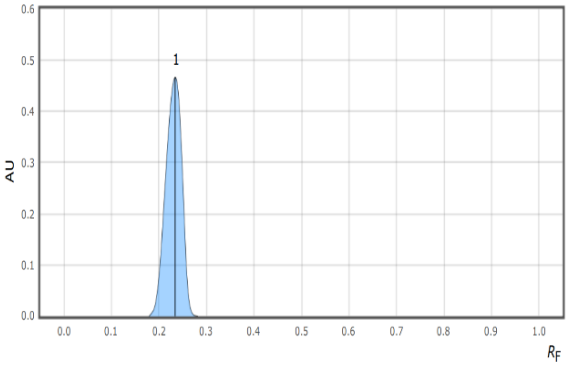

Fig 6d: Rosuvastatin calcium 4000 ng/ band

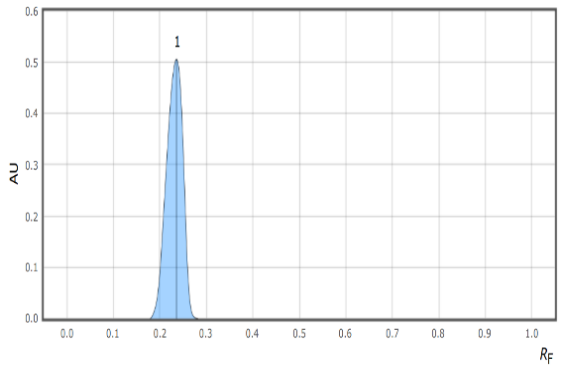

Fig 6e: Rosuvastatin calcium 5000 ng/ band

Figures 6a-6e depict the chromatograms of Rosuvastatin Calcium of different concentration range from 1000 – 5000 ng/ band.

3.4 Chromatogram of Ezetimibe

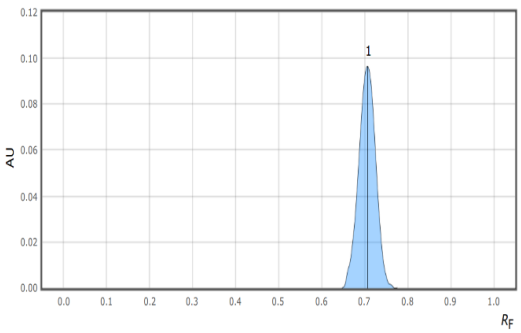

Fig 7a: Ezetimibe 500 ng/ band

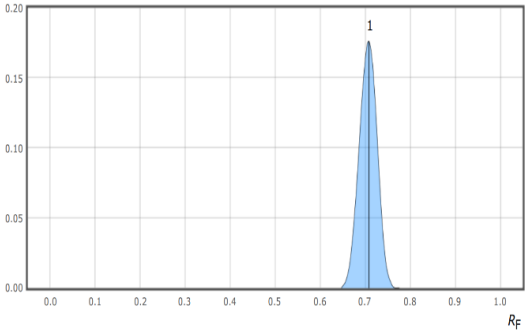

Fig 7b: Ezetimibe 1000 ng/ band

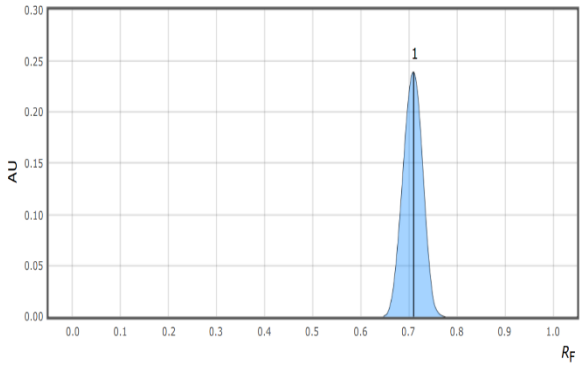

Fig 7c: Ezetimibe 1500 ng/ band

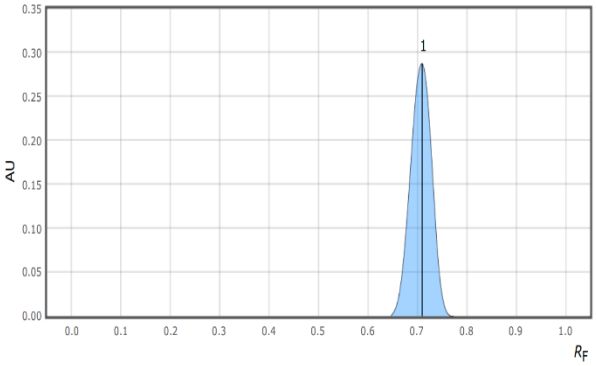

Fig7d: Ezetimibe 2000 ng/ band

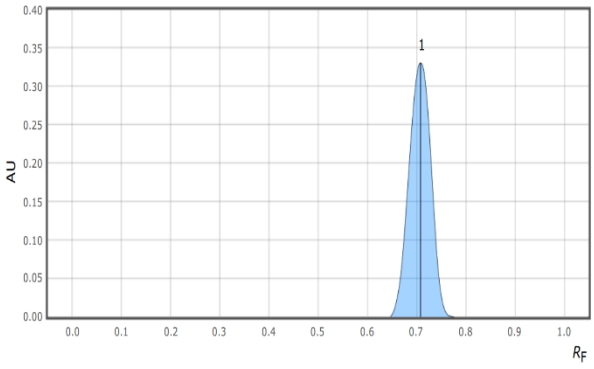

Fig 7e: Ezetimibe 2500 ng/ band

Figures 7a-7e depict the chromatograms of Ezetimibe of different concentration range from 500 – 2500 ng/ band.

3.5 Calibration plot of Rosuvastatin Calcium and Ezetimibe

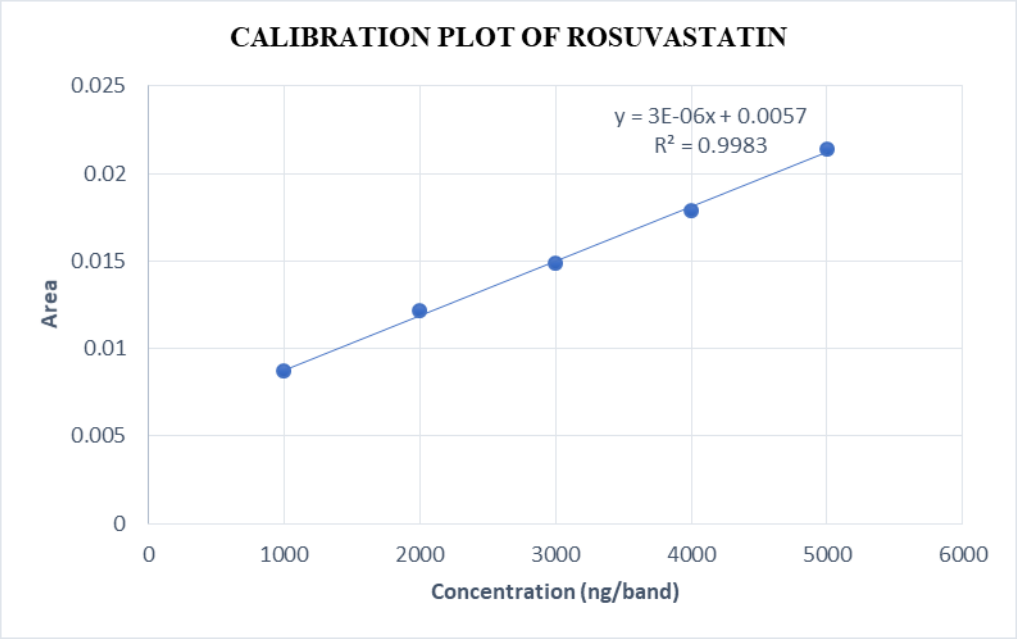

Fig 8a: Calibration Plot of Rosuvastatin calcium (Concentration v/s Peak area)

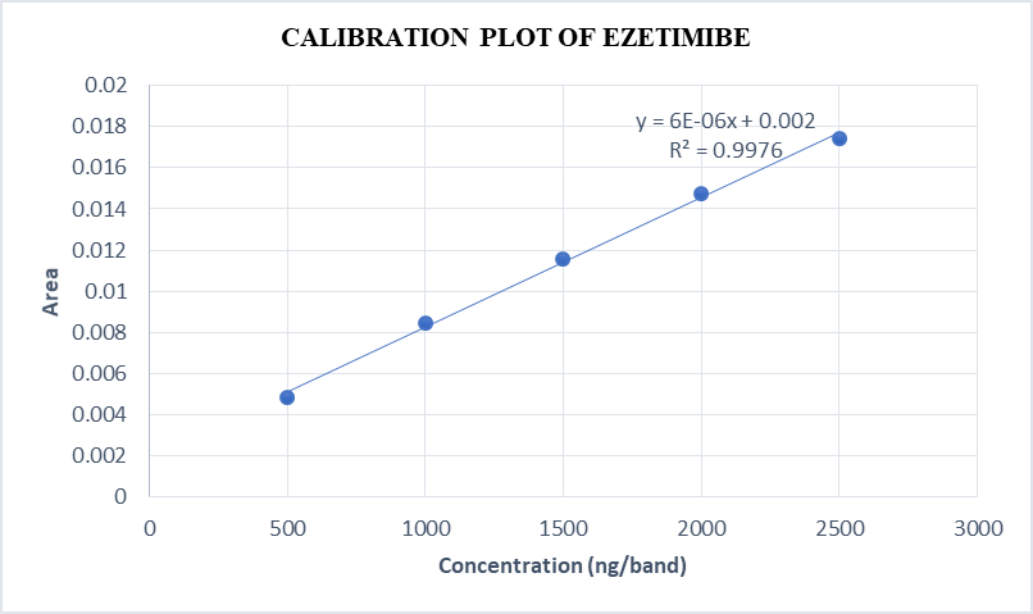

Fig 8b: Calibration Plot of Ezetimibe (Concentration v/s Peak area)

Figures 8a and 8b depict the calibration plot of Rosuvastatin Calcium and Ezetimibe in the concentration ranges of 1000 - 5000 ng/band and 500 - 1500 ng/band, respectively, against area.

3.6 Chromatogram of Standard drug mixture and Tablet mixture

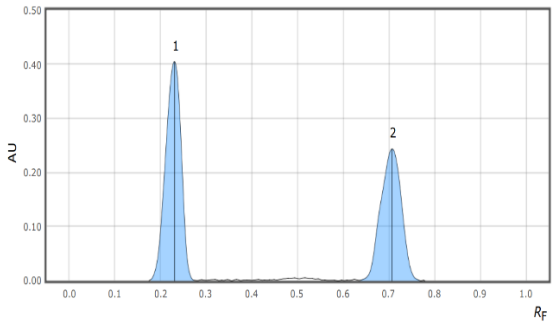

Fig 9a: Standard Drug Mixture (3 µL)

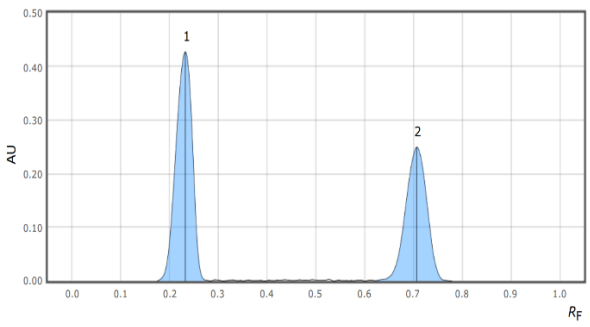

Fig 9b: Tablet mixture (3 µL)

Figures 9a and 9b represent the chromatograms of the standard drug mixture and tablet mixture in a concentration of 3000 ng/band for Rosuvastatin Calcium and 1500 ng/band for Ezetimibe.

3.7 Drug Content Per Tablet Determined by The Proposed Method

Table 2: Drug content per tablet

Drug	Area wise(mg)	Area wise (%w/w)
Rosuvastatin calcium	20.47	102.4
Ezetimibe	9.970	99.70

Table 2 shows the drug content and % label claim determined per tablet of the marketed formulation of ROZAVEL EZ.

3.8 Validation of the proposed method

The assay for the marketed formulation was established with the developed chromatographic conditions developed and it was found to be more accurate and reliable. The average drug content was found to be 102.40% for ROS and 99.70% for EZT of the labeled claim. The results are shown in table 2. Recovery studies were carried out to assess the accuracy of the method. These studies were carried out at three levels. The percentage recovery obtained was within the limits per ICH guidelines Q2(R2) and shown in Tables 3a and 4b. The precision of the method (Repeatability) was assessed by spotting 3µL of drug solution six times on a TLC plate, followed by the development of the plate and recording the peak area for 6 spots. The

% RSD for peak area values of Rosuvastatin Calcium was found to be 0.315% and for Ezetimibe was found to be 0.117%. The results are shown in Table 4c. The intermediated precision (The inter-day precision) was determined by analyzing standard solutions in the concentration range of 3000 ng/band for ROS and 1500ng/band for EZT for 3 days over one week. The % RSD obtained for inter-day precision was 0.115 and 0.194 for ROS and EZT, respectively. The results are shown in Table 5c. The Linearity for the detection of Rosuvastatin Calcium was 1000-5000 ng/band with a correlation coefficient (R^2) =0.998 and Ezetimibe was 500-2500ng/band with a correlation coefficient (R^2) = 0.997. The results are shown in table 6. Limit of Detection (LOD) and Limit of Quantification (LOQ were calculated by the method based on the standard deviation of response (a) and the slope of calibration plot (S), using the formulae LOD = 3.3σ/S and LOQ 10σ/S. The LOD was found to be 293.70ng/Spot and 128.16ng/Spot for ROS and EZT, respectively. Similarly, the LOQ was found to be 890.10ng/Spot and 388.38ng/Spot for ROS and EZT, respectively.

3.8.1 Accuracy

Table 3a: Recovery study results of Rosuvastatin Calcium

Level of Recovery	**Amount present (mg)**	**Amount Added (mg)**	**Drug recovered per tablet (mg)**	**Percentage label claim per tablet (%)**
			Area wise	**Area wise**
80 %	10	8	8.298	101.46
	10	8	8.308	101.58
	10	8	8.829	101.74
100 %	10	10	10.529	103.04
	10	10	10.274	102.74
	10	10	10.501	102.86
120 %	10	12	12.891	105.96
	10	12	12.882	105.84
	10	12	12.861	105.71

Table 3b: Recovery study results of Ezetimibe

Level of Recovery	Amount present (mg)	Amount Added (mg)	Drug recovered per tablet (mg)	Percentage label claim per tablet (%)
			Area wise	Area wise
80 %	5	4	4.099	101.38
	5	4	4.089	101.14
	5	4	4.086	101.06
100 %	5	5	5.269	103.37
	5	5	5.281	103.60
	5	5	5.265	103.29
120 %	5	6	6.343	105.19
	5	6	6.633	105.05
	5	6	6.335	105.35

Table 3c: Recovery Study –Statistical validation data of Rosuvastatin Calcium and Ezetimibe

Level of % Recovery	Mean % Recovery (n=3)		Standard Deviation (SD)		Relative Standard Deviation % RSD	
	Area wise		Area wise		Area wise	
	ROS	EZT	ROS	EZT	ROS	EZT
80	101.59	101.19	0.1400	0.1665	0.1382	0.1645
100	102.88	103.42	0.1509	0.1609	0.1509	0.1556
120	105.83	105.19	0.1250	0.1521	0.1181	0.1446

3.8.2 Precision

Table 4a: Data of Repeatability study

Sl. No.	Rosuvastatin calcium	Ezetimibe
	Peak Area	Peak Area
1	0.01652	0.01255
2	0.01643	0.01258
3	0.01651	0.01256
4	0.01647	0.01254
5	0.01656	0.01259
6	0.01654	0.01257

Table 4b: Result of Repeatability study

Amount Obtained per tablet (µg/ml)		**Drug Content per tablet (%)**	
Area wise		**Area wise**	
ROS	**EZT**	**ROS**	**EZT**
20.25	9.95	101.3	99.5
20.14	9.97	100.7	99.7
20.24	9.96	101.2	99.6
20.19	9.94	100.9	99.4
20.30	9.97	101.5	99.7
20.27	9.96	101.3	99.6

Table 4c: Repeatability study- Statistical validation

Method	**Drug**	**Mean (n=6)**	**Standard deviation**	**RSD (%)**
Area wise	**ROS**	101.15	0.31937	0.31574
	EZT	99.58	0.11690	0.11739

Table 5a: Inter-day Precision Data- Rosuvastatin calcium

Sl. No.	**DAYS (Concentration ng)**	**Peak Area**	**Drug recovered per tablet (mg)**	**Percentage label claim per tablet (%)**
1	**DAY 1**	0.01649	20.22	101.10
2		0.01652	20.25	101.25
3		0.01654	20.28	101.40
1	**DAY 2**	0.01651	20.24	101.20
2		0.01653	20.27	101.35
3		0.01655	20.29	101.45
1	**DAY 3**	0.01651	20.24	101.20
2		0.01654	20.28	101.40
3		0.01652	20.25	101.25

Table 5b: Inter-day Precision Data-Ezetimibe

Sl. No.	DAYS (Concentration ng)	Peak Area	Drug recovered per tablet (mg)	Percentage label claim per tablet (%)
1	**DAY 1**	0.01258	9.97	99.7
2		0.01257	9.97	99.7
3		0.01255	9.95	99.5
1	**DAY 2**	0.1259	9.98	99.8
2		0.1254	9.94	99.4
3		0.1257	9.97	99.7
1	**DAY 3**	0.1260	9.99	99.9
2		0.1257	9.97	99.7
3		0.1253	9.93	99.3

Table 5c: Inter-day Precision -Statistical validation

Concentration	Drug	Method	Mean (n=9)	SD	% RSD
3000/1500 (ng/band)	**Rosuvastatin calcium**	**Area**	101.28	0.11666	0.11518
	Ezetimibe		99.63	0.19364	0.19436

3.8.3 Linearity and Range

Table 6: Linearity and Range

Method parameters	Rosuvastatin calcium	Ezetimibe
	Area wise	**Area wise**
Linearity range (ng/spot)	1000-5000	500-2500
Slope	3E-06	6E-06
Intercept	0.0057	0.002
R^2 value	0.9983	0.9976

3.8.4 LOD and LOQ

Table 7: LOD & LOQ Results

Drug	Method	LOD (ng/spot)	LOQ (ng/spot)
Rosuvastatin calcium	Area wise	293.70	890.10
Ezetimibe	Area wise	128.16	388.38

4.0 SUMMARY AND CONCLUSION

In developed HPTLC, the Sample was dissolved in methanol. The mobile phase used in a ternary solvent system consists of Dichloromethane: Ethyl acetate: glacial acetic acid (8:1.5:0.5) The amount of drug present in the tablet lies within the IP limit. The developed method was validated as per ICH guidelines Q2R(2). The %RSD was found to be less than 2%, which indicates the developed is Precise. The developed method is cost-effective and utilizes fewer toxic solvents. Hence the developed method can be used for the routine analysis of Rosuvastatin Calcium and Ezetimibe in combined tablet dosage form.

The developed HPTLC method is simple, precise, and accurate. Compared to existing methods, the developed method uses simple reagents with minimal sample preparation procedures. HPTLC method has the advantages of short runtime, large sample capacity, and use of minimum volumes of solvents leading to environment friendly chromatographic procedure. The proposed method has the advantage of simplicity and convenience for the separation and quantitation of ROS and EZT in combination than existing methods and can be used for the assay of their dosage form. This method is accurate, precise, rapid, and selective for the simultaneous quantification of Rosuvastatin Calcium and Ezetimibe in tablet dosage form. Hence the developed HPTLC method can be conveniently adopted for routine analysis of above tablet dosage forms.

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6.0 CONFLICT OF INTEREST

There is no conflict of interest.

7.0 REFERENCES

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