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Stability Indicating Hptlc Method Development And Validation For Simultaneous Estimation Of Rosuvastatin Calcium And Ezetimibe In Bulk Form And Pharmaceutical Formulation

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Validated stability indicating high performance thin layer chromatography method has been developed for simultaneous estimation of Rosuvastatin calcium (ROS) and Ezetimibe (EZT) in bulk and pharmaceutical dosage form. The stationary phase consists of Silica Gel G 60 F254 and mobile phase consists of Toluene: Methanol: Ethyl acetate: Formic acid (8:2:1:0.01v/v/v/v) and detection was carried out at 245 nm. The R_f values of ROS and EZT were found to be 0.28 ±0.02 and 0.48 ±0.02 respectively. Validation of method was carried out as per ICH guidelines. Good linear relationship was found with the calibration curve for linearity in the concentration range of 300-800ng/spot and 300-800ng/spot for ROS and EZT with r^2 value 0.9997 and 0.999 respectively. For ROS and EZT the LOD was found to be 92ng/spot and 100ng/spot respectively. The LOQ value for ROS and EZT was found to be280ng/spot and 300ng/spot respectively. % recovery for ROS was found to be 98.46% to 102.1% and for EZT was found to be 99.6% to 100%. Both drugs have been exposed to the acidic, basic, neutral, oxidative, photolytic and thermal conditions for forced degradation studies. The degradation products obtained from degradation studies were resolved from the principle drug peak with different R_f values.

Keywords – HPTLC, Rosuvastatin calcium, Ezetimibe, validation, stability

INTRODUCTION

The combination of Rosuvastatin calcium(ROS) and Ezetimibe (EZT)is generally used in the treatment of hypercholesterolemia[1]. Chemically Rosuvastatin calcium (Fig 1) is bis [(E)7-[4-(4-fluorophenyl)-6-isopropyl-2[methyl-(methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxy hept-6-enoicacid] calcium salt[2-3].ROS is synthetic lipid lowering agent, it inhibits the HMGCOA reductase enzyme selectively and competitively. It reduces the risk of atherosclerosis which may leads to stroke, heart attack, peripheral vascular diseases and other cardiovascular complications. It is generally used for lowering the LDL cholesterol levels, total cholesterol, lipoprotein B and triglycerides in blood and increases the level of HDLcholesterol.[1]
Chemically Ezetimibe (Fig2) is (3R, 4S)-1-(4-fluorophenyl) - 3 - [(3S) - 3 - (4-fluorophenyl) -3-hydroxypropyl]-4- (4-hydroxyphenyl) azetidin - 2 - one. EZT act by selectively inhibiting the absorption of dietary cholesterol in intestine.[4-5] It inhibits the absorption of cholesterol without interfering with the absorption of fatty acids, bile acids, triglycerides and fat-soluble vitamins. EZT is used for the treatment of hyperlipidaemia. [1]
Literature survey for ROS and EZT were carried out individually, in combination with other drugs as well as in combination with each other. From literature survey of ROS it was found that there were different analytical methods were already reported. The reported analytical methods were UV spectrophotometric method[6-7], HPTLC method[8-9], HPLC methods[10] also stability indicating HPLC method.[8,11-12]There were also methods are available for quantitation of ROS with other drugs like finofibrate, amlodipine, clopidagrol and aspirin. Those methods were UV spectrophotometric[13-14], HPLC[15-17] and HPTLC[18-20]. From literature survey of EZT it was found that there were different analytical methods were already reported. The reported analytical methods were UV spectrophotometric method[21], HPTLCmethod[22], HPLC methods[23] also stability indicating HPLC method[24]. There were also methods available for quantitation of EZT with other drugs like simvastatin, lovastatin, finofibrate, atorvastatin and valsartan. Those methods were UV spectrophotometric[25-26], HPLC[27-29]and HPTLC[30]. There were UV spectrophotometric[31], HPTLC[32], HPLC[33-

[34]and stability indicating HPLC methods[35-36] reported for analysis of ROS and EZT in combination. So, from the literature survey it was found that there was no any stability indicating HPTLC method was developed for simultaneous estimation of ROS and EZT in bulk form as well as in pharmaceutical dosage form.

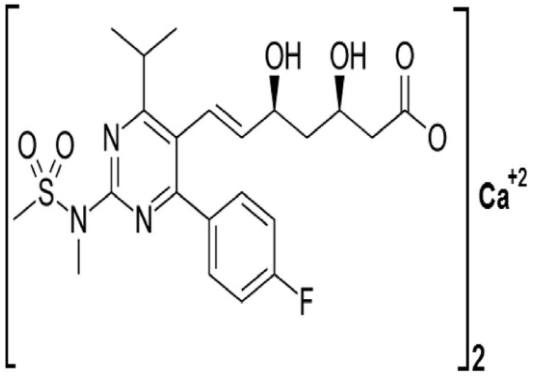

FIG 1: ROSUVASTATIN CALCIUM

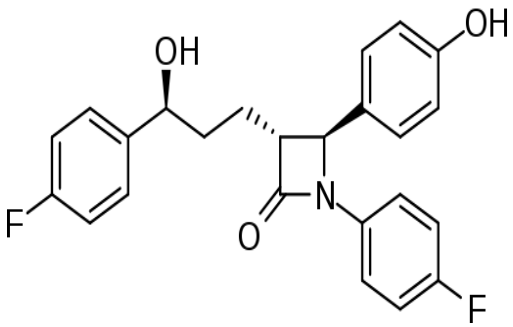

FIG 2: EZETIMIBE

MATERIALS AND METHODS:

Chemicals and Reagents: Gift samples of ROS and EZT were provided by Apotex Pharmaceuticals Pvt. Ltd and Dr. Reddys labs, Hyderabad respectively. The marketed formulation that is RAZEL EZ tablet with dose of 10mg each ROS and EZT was procured from local market, Pune. Analytical grade solvents and TLC aluminium plates pre-coated with silica gel 60 F254 used for this study were purchased from Merck Pvt. Ltd., Mumbai.

Instrumentation and Chromatographic Conditions: TLC aluminium plates pre-coated with silica gel 60 F254 (20 × 20) with 250 µm thickness were used for chromatographic separation of drugs. Hamilton microliter syringe (100 µl) was used for sample application on the plate in the form of 6mm bands. Development was carried out by linear ascending manner in 10 × 10 cm twin trough glass chamber (CAMAG) by using Toluene: Methanol: Ethyl Acetate: Formic Acid (8:2:1:0.01v/v/v/v) as mobile phase. Saturation time for mobile phase was optimized to 30 minutes. The length of chromatogram run was 8 cm. Plates were air dried after development and scanned at 245nm by using Camag TLC Scanner equipped with win CATS software version 1.4.4.6337. Deuterium lamp was used as source of radiation and slit dimension was kept at 5 × 0.45 mm.

PREPARATION OF SOLUTIONS

Preparation of standard stock solution: 10mg of each ROS and EZT were weighed accurately and transferred in to separate 10ml volumetric flask and the volume was made up to the mark with methanol to get standard stock solution of ROS R1 (1000µg/ml) and EZT E1(1000µg/ml).

Preparation of working standard solution: 1ml of standard stock solution of each ROS R1(1000µg/ml) and EZT E1(1000µg/ml) were transferred in to separate 10 ml volumetric flask and volume was made up to the mark with methanol to get working standard solution of each ROS R2 (100µg/ml) and EZT E2 (100µg/ml).

Preparation of mixed standard solution: 5 ml working stock solution of each ROS R2 (100µg/ml) and EZT E2 (100µg/ml) were mixed together to get mixed standard stock solutions.

OPTIMIZATION OF MOBILE PHASE

The main objective in developing the stability indicating HPTLC method is to achieve the better resolution of ROS and EZT and from its degradation product. The optimized mobile phase was Toluene: Methanol: Ethyl acetate: Formic acid (8:2:1:0.01v/v/v/v) and the development was carried out in 10 cm × 10 cm twin trough glass chamber by linear ascending method and detection was done at 245nm. The retention factors were observed to be 0.28±0.02 for ROS and 0.48±0.02 for EZT. Representative densitogram of mixed standard solution of ROS and EZT is shown in Fig. 3.

METHOD VALIDATION

Validation of method has been done as per ICH guidelines Q2 (R1) for linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and specificity[37].

Linearity: For linearity the mixed standard solution containing ROS (100µg/ml) and EZT (100µg/ml) was applied on TLC plate in range 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8µl for each ROS and EZT. The same procedure was repeated for 3 times (n=3). The calibration curve was obtained by plotting peak areas vs. concentration shown in Table 1and Fig 4and 5.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The limit of detection and quantification were calculated based on the equations as per ICH guidelines. Calculated values of LOD and LOQ are given in Table 2.
LOD= 3.3(SD)/S
LOQ=10(SD)/S

Precision: Intraday and interday precision studies were carried out by applying 3 spots of mixed standard solution containing ROS (100µg/ml) and EZT (100µg/ml) on TLC plate of 0.4, 0.6 and 0.8 µl for each ROS and EZT for three times a different time intervals on same day for intra-day precision and on 3 different days for inter day precision. The percentage RSD was calculated by reporting areas. The results are shown in Table 3and 4.

Repeatability: Repeatability of sample application was assessed by applying 600 ng/spot of working standard solution on TLC plate(n=6). The plate was run with mobile phase Toluene: Methanol: Ethyl Acetate: Formic Acid (8:2:1:0.01 v/v/v/v). The plate was dried and scanned at 245 nm and densitograms were recorded and areas were reported. This procedure was repeated 6 times. The repeatability of method was evaluated by %RSD. The results are shown in Table 5.

Robustness: By making small deliberate changes in optimized conditions like saturation time ±5 minutes, wavelength ±1nm, mobile phase composition ± 0.5 ml robustness of the method was accessed. 600 ng/spot of working standard solution were spotted on TLC plate. The developed plate was dried and scanned at 245 nm. Densitograms were recorded and % RSD was calculated. The results are given in Table 6.

Recovery study: The accuracy of method was done by applying the optimized method to the marketed formulation of combination of ROS and EZT that is the Razel EZ tablet which contains 10mg of ROS and 10 mg of EZT. To this formulation known amount of ROS and EZT corresponding to 80, 100 and 120% of label claim has been added. The % recoveries were calculated and given in table 7.

Assay: For determination of content of ROS and EZT in marketed formulation, the tablet containing 10mg of ROS and 10mg of EZT. 20 tablets were weighed accurately and average weight was calculated. The amount equivalent to one tablet was weighed and transferred to 100ml volumetric flask and dissolved in methanol and the volume was made up to the mark with methanol to get the concentration of 100 µg/ml of each ROS and EZT. The solution is then filtered by using whatman filter paper to remove any undissolved excipients. The spots of 4 µl of prepared solution and mixed standard solution containing 100µg/ml of each ROS and EZT were applied on TLC plate. The developed plate was dried and scanned at 245 nm and densitogramwas recorded. The areas and R_f values were recorded. The drug content was calculated and reported in table 8.

FORCED DEGRADATION STUDIES:

Forced degradation studies were carried out by applying stressed conditions like acidic, basic, oxidative, neutral, thermal and photolytic to the drugs by controlling the stress agents, time of exposure, and concentration. The acceptable degradation is 5-20%. This ensures that the peak of degradation product does not interfere with the peak of principle drug peak. These studies were carried out as per ICH guidelines[38].

Acid Degradation: Mixture of 5ml mixed standard solution and 5ml of 0.1N HCL was refluxed at 60° for 2 hrs and cooled. This mixture was characterized by applying the developed validated method. Densitogram was recorded as shown in Fig 6 and details of acid degradation study are given in Table 9.

Base Degradation: Mixture of 5ml mixed standard solution and 5ml of 0.1N NaOH was refluxed at 60° for 2 hrs and cooled. This mixture was characterized by applying the developed validated method and densitogram was recorded and % degradation was calculated by areas. Recorded densitogramis shown in Fig7 details of base degradation study are given in Table10.

Neutral Degradation: A mixture of 5ml mixed standard solution and 5ml of Distilled water was kept at room temperature for 8 hrs. The resultant solution was characterized by applying the developed validated method. Densitogram was recorded as shown in Fig 6and details of neutral degradation study are given in Table 3.

Oxidative Degradation: A mixture of 5ml mixed standard solution and 5ml of 6% Hydrogen Peroxide was kept at room temperature for 6 hrs. The resultant degraded solution was characterized by applying developed validated method. Densitogram was recorded as shown in Fig 7 and details of oxidative degradation study are given in Table 4.

ThermalDegradation:10 mg of ROS and 10 mg of EZT powder was kept in hot air oven at 60˚C. Powdered sample was withdrawn after 8 hrs and solution was prepared. The solution was characterized by applying developed validated method. Densitogram was recorded as shown in Fig 8 and details of thermal degradation study are given in Table 5.

Photolytic Degradation: The photolytic stability of the drug was also studied by exposing the drug powder to direct sunlight for 12 hrs. The powder was withdrawn and working standard solution was prepared from it. The standard solution was characterized by developed validated method. Densitogram was recorded as shown in Fig 9 and details of acid degradation study are given in Table 6.

RESULTS AND DISCUSSION:

Optimization of Mobile Phase: The optimized mobile phase was Toluene: Methanol: Ethyl acetate: Formic acid (8:2:1:0.01v/v/v/v). The retention factors were observed to be 0.28±0.02 for ROS and 0.48±0.02 for EZT. The representative densitogram for mixture containing ROS and EZT is shown in Fig 3

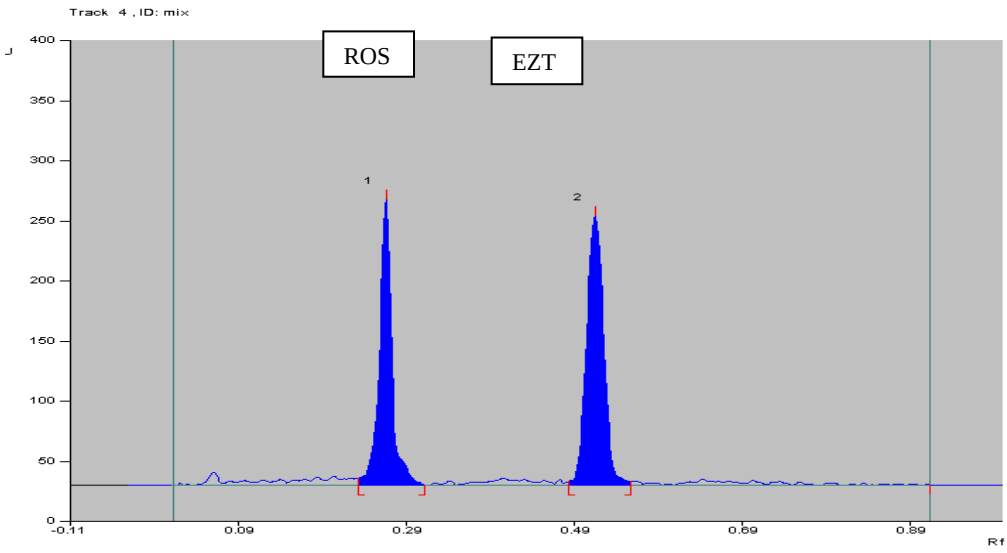

FIG 3: REPRESENTATIVE DENSITOGRAMS OF ROS AND EZT

METHOD VALIDATION

Linearity: The linear regression data for the calibration curves (n=6) showed good linear relationship over the concentration range of 300 - 800ng/spot for ROS (r2=0.9997) and 300-800ng/spot for EZT (r2=0.999). The calibration

curve of ROS and EZT was obtained by plotting area vs concentration (Fig 4, 5) and the details of linearity are given in Table 1

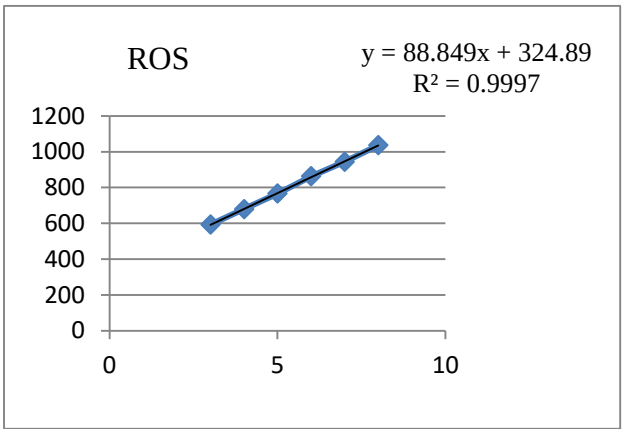

FIG 4: LINEARITY GRAPH OF ROS

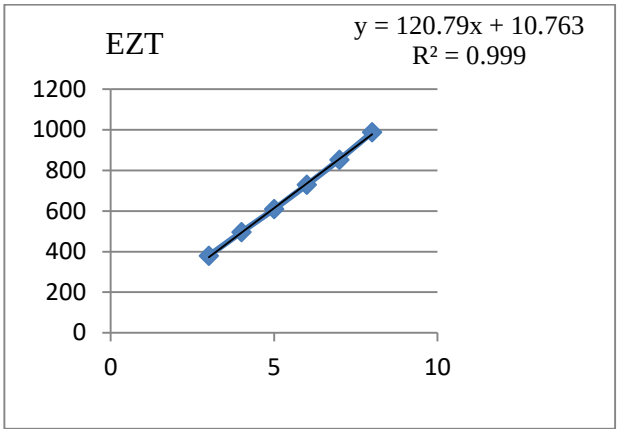

FIG 5: LINEARITY GRAPH OF EZT

TABLE 1: REGRESSION DETAILS OF ROS AND EZT

Parameter	ROS	EZT
Linearity ng/spot	300-800ng/spot	300-800ng/spot
R2	0.9997	0.999
Slope	88.849	120.79
Intercept	324.89	10.763

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

TABLE 2: LOD AND LOQ DETAILS OF ROS AND EZT

DRUG	LOD(ng/spot)	LOQ(ng/spot)
ROS	92	280
EZT	100	300

Precision: The Precision of the method was verified by and intra-day and inter-day precision with nine replicate analysis of working standard solution of ROS and EZT. The developed method was found to be precise as the %RSD values for intra-day and inter-day precision studies were not less than 2% as recommended by ICH guidelines and given in table 3 and 4.

TABLE 3: INTRA-DAY PRECISION DETAILS OF ROS AND EZT

Drug	Conc. (ng/spot)	Avg. Area (n=3)	% RSD
ROS	400	753.8	1.73
	600	878.3	0.84
	800	1063.7	1.56
EZT	400	573.9	1.40
	600	721.9	0.63
	800	1009.3	0.06

TABLE 4: INTER-DAY PRECISION DETAILS OF ROS AND EZT

Drug	Conc. (ng/spot)	Avg. Area (n=3)	% RSD
ROS	400	764.5	0.41
	600	877.4	0.79
	800	1046.2	0.90
EZT	400	528.6	1.42
	600	835.8	0.86
	800	1052.7	1.02

Repeatability: Repeatability of the method was checked by analyzing the mixed standard solution, by applying spots of 6µl on TLC Plate (n=6). %RSD was found to be less than 2% which was in the acceptable range and results are given in the table 5 for ROS and EZT respectively.

TABLE 5: REPEATABILITY STUDY OF ROS AND EZT

Drug	Conc.(ng/spot)	Area (n=3)	%RSD
ROS	600	878.3	0.84
EZT	600	721.9	0.63

Robustness: Small, deliberate changes were made in optimized conditions like mobile phase composition, saturation time and wavelength (n=3). The acceptance criterion for %RSD was found to be less than 2% which was within the accepted range. The results of robustness studies are given in the following Table 6.

TABLE 6: ROBUSTNESS DETAILS OF ROS AND EZT

Conditions	$R_f$value		Avg. Area (n=3)		%RSD	
	ROS	EZT	ACY	EZT	ROS	EZT
Mobile Phase Composition (± 0.5ml Toluene)						
Toluene :Methanol: Ethyl acetate: Formic Acid (7.5:2:1:0.01v/v/v/v)	0.30	0.50	663.06	833.5		
Toluene :Methanol: Ethyl acetate: Formic Acid (8:2:1:0.01v/v/v/v)	0.28	0.48	653.8	848.5	1.74	1.29
Toluene :Methanol: Ethyl acetate: Formic Acid (8.5:2:1:0.01v/v/v/v)	0.26	0.49	676.8	854.7		
Wavelength (± 1 nm)						
249 nm	0.28	0.49	973.9	779	0.56	1.46
245 nm	0.29	0.50	978.2	763.8		
246 nm	0.27	0.46	967.4	757.1		
Duration of Saturation (± 5min)						
25	0.30	0.68	778.5	867.5	1.54	1.01
30	0.28	0.47	754.9	872.6		
35	0.29	0.49	768.3	884.		

Recovery study: The % recoveries were found to be 98.46% to 102.1% for ROS and 99.6% to 100% for EZT in Razel EZ tablet. The % recovery values obtained are mentioned in Table7.

TABLE 7: RECOVERY STUDY OF ROS AND EZT

Drug	% level	Initial amount added(mg)	Amount added (mg)	%Recovery
ROS	80	10	8	98.46
	100	10	10	102.1
	120	10	12	98.95
EZT	80	10	8	100
	100	10	10	99.81
	120	10	12	100

Assay: The peaks of ROS and EZT were observed at Rf 0.28 and 0.49 in chromatogram without any interference of excipients which are present in the formulation. The drug content was found to be 99.18% for ROS and 98.98% for EZT which is given in table 7.

TABLE 8: ASSAY STUDIES OF ROS AND EZT

DRUG	$R_f$value	Drug Content (%)	Mean %
ROS	0.29	98.78	99.18
		99.20	
		99.56	
EZT	0.50	98.63	98.98
		99.05	
		99.26	

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FORCED DEGRADATION STUDIES:

Acid Degradation: The densitogram of acid degradation study (Fig.6) showed peaks of ROS and EZT at R_f 0.29 and 0.49 respectively. The details of acid degradation are given in Table 9.

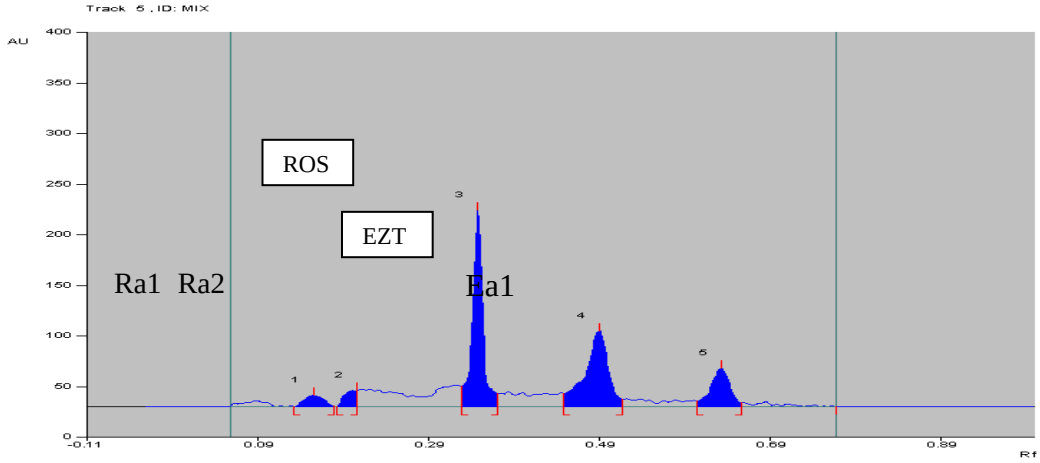

FIG 6: ACID DEGRADATION OF ROS AND EZT

TABLE 9: DETAILS OF ACID DEGRADATION STUDY OF ROS AND EZT

S NO.	Peak	R_f value	% degradation
1.	Std ROS	0.35	-
2.	Std EZT	0.49	-
3.	Degradants peak(Ra1)	0.15	4.59
4.	Degradants peak(Ra2)	0.20	4.20
5.	Degradants Peak(Ea1)	0.63	15.04

Base Degradation: The densitogram of base degradation study (Fig7) showed peaks of ROS and at R_f0.30 and 0.49 respectively. It was found that the degradant peaks were separated from the drug peak. The details of base degradation are given in table 10.

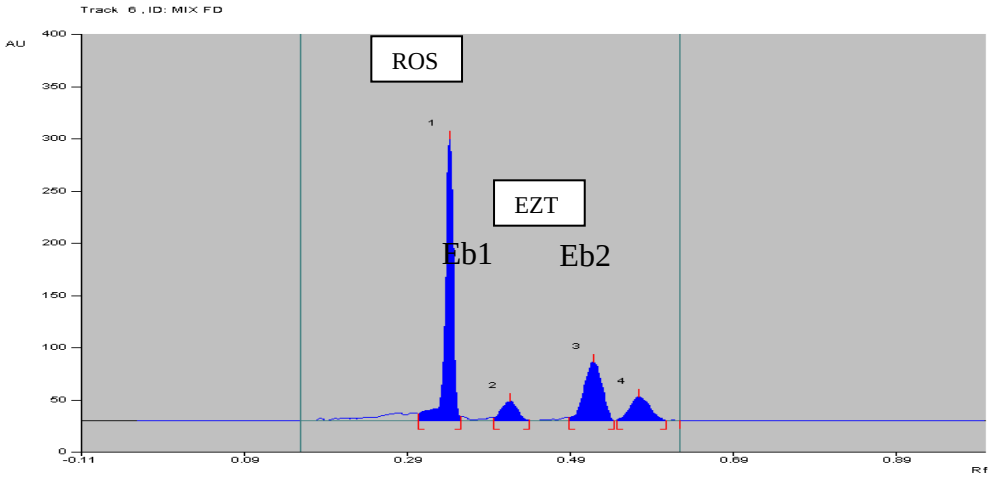

FIG 7: BASE DEGRADATION OF ROS AND EZT

TABLE 10: DETAILS OF BASE DEGRADATION STUDY OF ROS AND EZT

Peak	$R_f$value	% degradation
Std ROS	0.30	-
Std EZT	0.49	-
Degradants peak(Eb1)	0.42	7.80
Degradants Peak(Eb2)	0.57	11.88

Neutral Degradation: The densitogram of neutal degradation study (Fig.8) showed peaks for degradation of ROS and EZT at R_f 0.26 and 0.47 respectively. There were no degradation peaks observed for ROS and EZT in mixture. Details of neutral degradation are given in Table 11

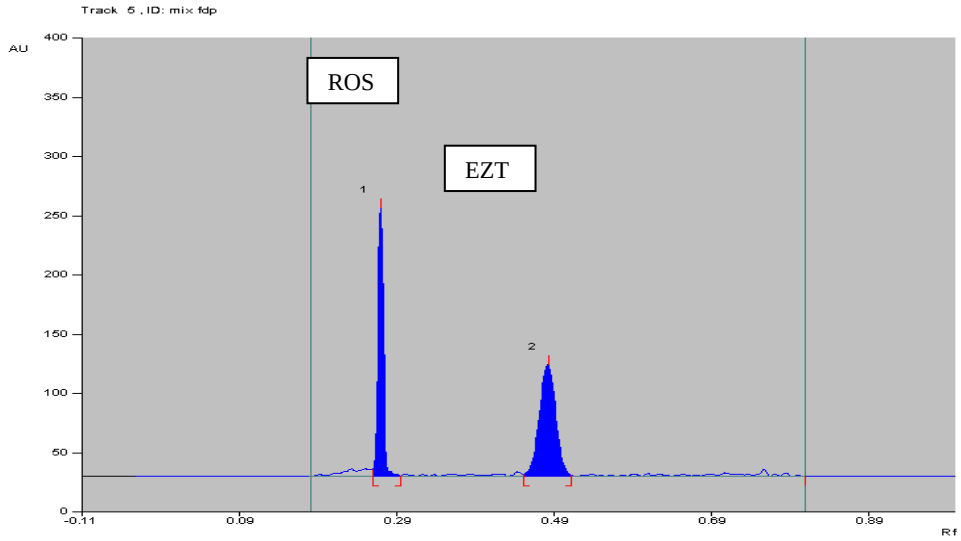

FIG 8: NEUTRAL DEGRADATION OF ROS AND EZT

TABLE 11: DETAILS OF NEUTRAL DEGRADATION STUDY OF ROS AND EZT

Peak	R_f value	% degradation
Std ROS	0.26	-
Std EZT	0.47	-

Oxidative degradation: The densitogram of oxidative degradation study (Fig.8) showed peaks for degradation of ROS and EZT in mixture showed peaks at R_f0.28and 0.47 respectively. Details of oxidative degradation are given in Table 12.

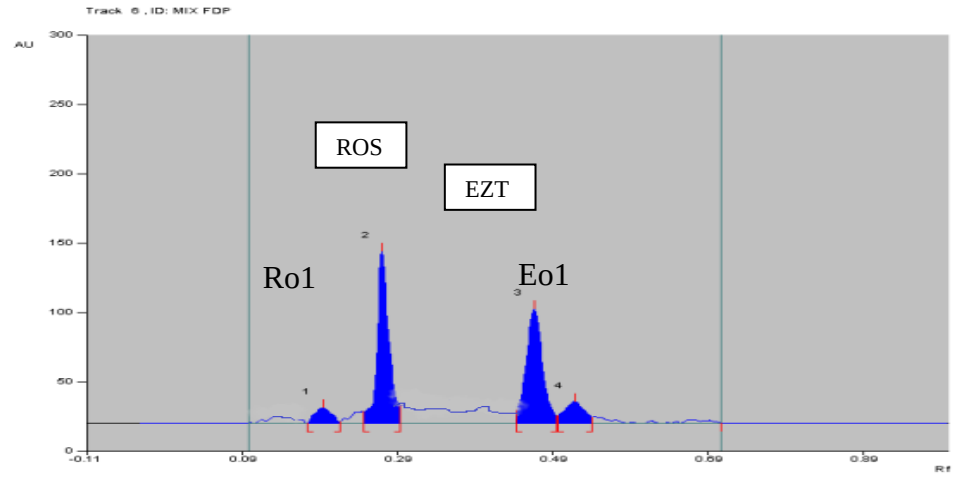

FIG 9: OXIDATIVE DEGRADATION OF ROS AND EZT

TABLE 12: DETAILS OF OXIDATIVE DEGRADATION STUDY OF ROS AND EZT

Peak	$R_f$value	% degradation
Std ROS	0.28	-
Std EZT	0.47	-
Degradants peak(Ro1)	0.19	5.88
Degradants Peak(Eo2)	0.52	9.60

Thermal degradation: The densitogram of thermal degradation study (Fig.10) showed peaks for degradation of ROS and EZT at R_f 0.29 and 0.50 respectively. Details of thermal degradation are given in Table 13.

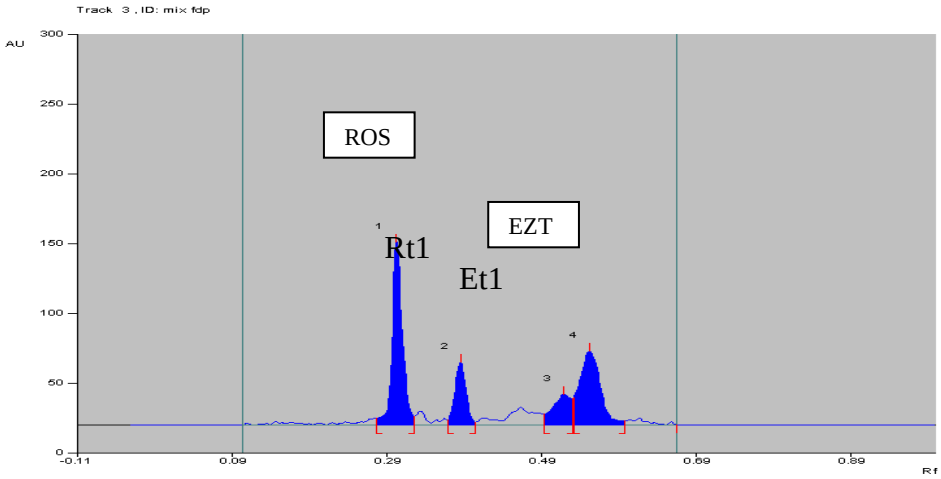

FIG 10: THERMAL DEGRADATION OF ROS AND EZT

TABLE 13: DETAILS OF OXIDATIVE DEGRADATION STUDY OF ROS AND EZT

Peak	R_f value	% degradation
Std ROS	0.29	-
Std EZT	0.50	-
Degradants peak(Rt1)	0.38	10.24
Degradants Peak(Rt2)	0.48	9.03

Photolytic degradation: The densitogram of photolytic degradation study (Fig.11) showed peaks for degradation of ROS and EZT at R_f 0.26 and 0.49 respectively**.** Details of thermal degradation are given in Table 14.

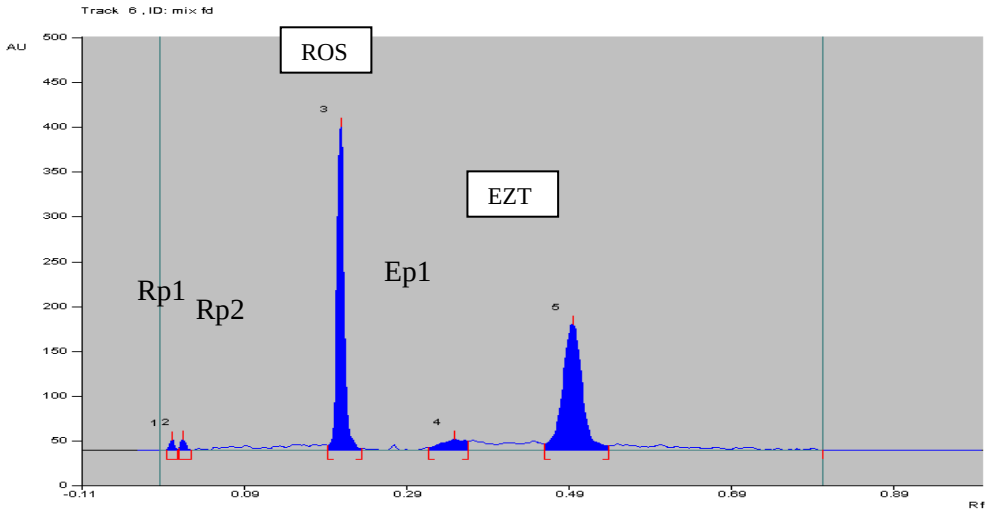

FIG11: PHOTOLYTIC DEGRADATION OF ROS AND EZT

TABLE 14: DETAILS OF PHOTOLYTIC DEGRADATION STUDY OF ROSAND EZT

Peak	R_f value	% degradation
Std ROS	0.26	-
Std EZT	0.49	-
Degradants peak(Rp1)	0.00	0.90
Degradants Peak(Rp2)	0.01	1.10
Degradants Peak(Ep1)	0.35	5.35

CONCLUSION:

The simple, rapid, selective, accurate, precise and robust stability indicating validated HPTLC method has been developed for simultaneous estimation of ROS and EZT in pharmaceutical dosage form. The optimized mobile can separate the drugs with better resolution; it can also separate the degradant peaks from drugs peaks with better resolution when force degradation studies were carried out. The force degradation study and validation was carried out as per ICH guidelines. The method is environment friendly as it requires less consumption of solvents. It requires shorter time for analysis.

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REFERENCES

1. Vavlukis M and Vavlukis A:Adding ezetimibe to statin therapy latest evidence and clinical implications: Drugs in context 2018;vol 7.
2. Indian Pharmacopoeia: The Indian Pharmacopoeia commission2007; 3:1676-1678.
3. https://www.drugbank.ca/drugs/DB01098
4. The Merck Index: An encyclopedia of chemical drugs and biological. 14th edition. USA. Merck research laboratories2006;578- 670.
5. https://www.drugbank.ca/drugs/DB00973
6. Alka G, Mishra P and Shah K: Simple UV Spectrophotometric Determination of Rosuvastatin Calcium in Pure Form and in Pharmaceutical Formulations. E-Journal of Chemistry 2009; 6(1): 89-92.
7. Sailaja1 B, Sravana Kumari K: Analytical Method Development and Validation for the Estimation of Rosuvastatin Calcium in Raw Material and Tablet Formulation by UV Spectrometric Method. Saudi Journal of Medical and Pharmaceutical Sciences2016; Vol-2:7-11.
8. Hasumati AR, Sadhana JR, Jayant BD and Chaggan NP: Development and Validation of Two Chromatographic Stability-Indicating Methods for Determination of Rosuvastatin in Pure Form and Pharmaceutical Preparation. International Journal of Chem Tech Research 2009;1: 677-689.
9. Fathalla FB, Fawzia I, Alaa K and Tarek E: Stability Indicating TLC Method for The Determination of Rosuvastatin and Identification of Some Degradation Products Using Electrospray Ionization Mass Spectrometry. Journal of Liquid Chromatography and Related Technologies2013;1-36.
10. Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT and Shah AK: A New Improved RP-HPLC Method for Assay of Rosuvastatin Calcium in Tablets. Indian Journal of Pharmaceutical Sciences2010;72 (5): 592-598.
11. Balanagamani D, Deepthi K, Sivasubramanian KL: Development and validation of stability indicating RP-HPLC methods for Rosuvastatin Calcium. International research journal of pharmacy 2016; 7(12): 118-123.
12. Mohamed El-Kassem M Hassouna, Hafsa Omar Salem: Stability Indicating New Rp-HPLC Method for The Determination Of Rosuvastatin Calcium in Pure and Tablets Dosage Forms. International journal of Applied Pharmaceutical and Biological Research 2017; 2(2):11-27.
13. 13.SevdaRR, Ravetkar AS, Shirote PJ: UV Spectrophotometric estimation of Rosuvastatin Calcium and Fenofibrate in bulk Drug and Dosage Form using Simultaneous Equation Method: International Journal of Chem Tech Research2011; Vol. 3 No.2: 629-635.
14. 14.Karunakaran A, Vetsa S, Chinthala R, Jayamaryapan M: Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by UV-Spectrophotometry and RP-HPLC. Stamford Journal of Pharmaceutical Science 2011;4(1): 58-63.
15. 15.Muhammad A, Tazeem A, Ghulam M, Muhammad D, Syed NR, Muhammad F N: Simultaneous Estimation of Rosuvastatin and Amlodipine in Pharmaceutical Formulations using Stability Indicating HPLC method.Brazilian Journal of Pharmaceutical Sciences2014;vol. 50:629-638.
16. Sumalatha M, Haritha PK: Analytical method development and validation for the simultaneous estimation of Rosuvastatin and Finofibate in Tablet Dosage form by Reverse Phase High Performance Liquid Chromatography. Indian Journal of Research in Pharmacy and Biotechnology 2013; 850-856.
17. Dipali T, Raurale AM, Bharande PD, Mali AN, Gadkari AV, Bhosale VR: Development and validation of a RP-HPLC-PDA Method for Simultaneous Determination of Rosuvastatin calcium and Amlodipine besylate in Pharmaceutical Dosage Form. Journal of Chemical and Pharmaceutical Research 2012; 4(5):2789-2794.

18. Potawale RS, Gabhe SY: HPTLC Method For Simultaneous Determination of Rosuvastatin and Fenofibrtae in Bulk and Pharmaceutical Formulation. International Journal of Pharmacy and Pharmaceutical Sciences2014; 6:323-326.
19. Purkar AJ, Balap AR, Sathiyanarayanan L, Mahadik KR: Development and Validation of HPTLC Method for Simultaneous Determination of Rosuvastatin Calcium and Aspirin in Its Pure and Pharmaceutical Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences2014; 6: 704-706.
20. Agrawal P, Rao JR and Doke C: Development And Validation Of Stability Indicating HPTLC Method for Simultaneous Estimation of Aspirin, Clopidogrel Bisulphate and Rosuvastatin Calcium in Bulk and Pharmaceutical Dosage Formulation. World Journal Of Pharmacy and Pharmaceutical Sciences 2018; 7:930-953.
21. Sudha Lakshmi PB, Ramchandran D. and Rambabu C: Spectrophotometric Determination of Ezetimibe. E-Journal of Chemistry 2010;7(1): 101-104.
22. Mahadik MV, Dhaneshwar SR: Application of a Stability-Indicating HPTLC Method for the Quantitative Determination of Ezetimibe in pharmaceutical dosage forms. Asian Journal of Pharmaceutical Sciences 2007; 2 (5): 182-190.
23. Baokar SB, Erande RS. and Shaikh SG: Analytical Method Development and Validation for Estimation of Ezetimibe from Tablet Dosage Form by Using RP-HPLC. International Journal of Research in Pharmaceutical and Biomedical Sciences2011;2 (2): 833-841.
24. Kumar P, Ahmad Y and Ghosh A: A stability indicating RP-HPLC method development for Determination of Ezetimibe in Tablet Dosage Form. Der Pharma Chemica 2012; 4 (3):1296-1304.
25. 25.Kondawar MS, KambleKG, Maharshi KH, Khandare MM: UV Spectrophotometric estimation of Ezetimibe and Fenofibrate in Bulk drug and Dosage form using Simultaneous Equation Method. International Journal of ChemTech Research 2011;3(2):749-754.
26. Jain N, Jain R, Swami H, Pandey S and Jain K, Spectrophotometric Method for Simultaneous Estimation of Simvastatin and Ezetimibe In Bulk Drug and Its Combined Dosage Form. International Journal of Pharmacy and Pharmaceutical Science 2009; 1: 170-175.
27. Qutab SS, Razzaq SN, Khan IU, Ashfaq M and Shuja ZA: Simultaneous Determination of Atorvastatin Calcium and Ezetimibe in Pharmaceutical Formulations by Liquid Chromatography. Journal of Food and Drug Analysis 2007; 15 (2):139-144.
28. Ramachandran S, Mandal BK and Navalgund SG: Stability-Indicating HPLC Method for the Simultaneous Determination of Valsartan and Ezetimibe in Pharmaceuticals. Tropical Journal of Pharmaceutical Research2014; 13 (5): 809-817.
29. Khana IU, Kausara T, Ashfaqb M And Sharifa S: Development and Validation of Liquid Chromatographic Method for the Simultaneous Estimation of Ezetimibe and Lovastatin in Human Plasma.J. Chil. Chem. Soc 2010; 55(4): 461-464.
30. Stephen Rathinaraj B, Rajamanickam V, Rajveer C, Kumaraswamy D, Shehraobanglae G, Arunachalam A: Development and Validation of a HPTLC Method for the Estimation of Simvastatin and Ezetimibe. International Journal of Pharmaceutical & Biological Archives 2010; 1(4): 325 – 330.
31. Gunge A, Deshpande P: Chemometric-Assisted UV Spectrophotometric Method for Determination of Rosuvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Form. International Journal Of Pharmacy and Pharmaceutical Research 2018; 11:248-258.
32. Sharma MC, Sharmaa S, Kohli DV, Sharma AD.: 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): 1-7.
33. Ramu K, Aleti P, Venisetty RK: Analytical Method Development and Validation Of Simultanious Estimation of Ezetamibe and Rosuvastatin in Tablet Dosage Form by Rp-HPLC. International Journal of Pharmacy and Biological Sciences 2013;3(4): 343-353.
34. Eswarudu MM, Mounica P, Venkatesh D, Nagalakshmi B: Method Development And Validation For Simultaneous Estimation Of Rosuvastatin Calcium And Ezetimibe In Pharmaceutical Dosage Form By Rp-HPLC. Iternational Research Journal of Pharmaceutical and Applied Sciences2012; 2(1):24-36.
35. Anuradha KG and Shah VD: 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 2020; 5(3): 280-298.
36. Hemant KT and Gowri Sankar D, Simultaneous estimation of rosuvastatin calcium and ezetimibe in pharmaceutical formulation by RP-HPLC method with forced degradation studies. Der Pharmacia Lettre2018; 8 (6):288-298.
37. Validation of Analytical Procedures, Proceedings of the International Conference on Harmonisation (ICH), Geneva. Commission of the European Communities 1996.
38. Blessy M, Ptel RD, Prajapati PN, Agrawal YK: Development of Forced Degradation and stability indicating studies of drugs - A Review. Journal of Pharmaceutical Analysis 2020; 4(3):159-165.