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A validated HPTLC method for determination of simultaneous estimation Rosuvastatin Calcium and Ezetimibe in pharmaceutical solid dosage form

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Abstract

This paper Described validated high performance liquid chromatographic (HPTLC) method for estimation of Rosuvastatin Calcium (ROS) and Ezetimibe (EZE) in tablet dosage form. The method involved separation of components by TLC on a precoated silica gel 60 F_{254} using a mixture of n-butanol: methanol (3:1) as a mobile phase. Detection of spots was carried out at 274 nm and 230 nm for Rosuvastatin Calcium and Ezetimibe combinations, respectively. The mean retardation factor for Rosuvastatin Calcium and Ezetimibe were found to be 0.90 ±0.01, 0.82±0.05, respectively. The linearity and range was 0.1 to 0.5 µg/spot for two drugs. The method was validated for precision, accuracy and reproducibility

Keywords: HPTLC, Rosuvastatin Calcium, Ezetimibe.

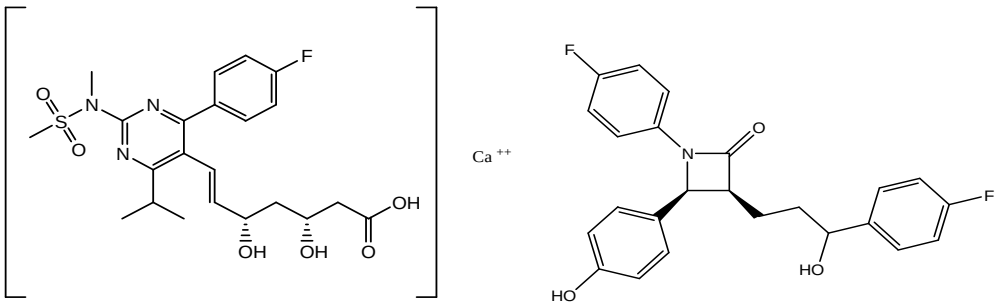
Introduction

Rosuvastatin calcium is chemically (3*R*, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(*N*-methyl methane sulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid.it is a competitive inhibitor of the enzyme HMG-CoA reductase[1], the rate –limiting enzyme that converts 3-hydroxy -3-methylglutaryl coenzyme A to mevalonate, precursor for cholesterol. It is a cholesterol lower agents. In recent years some HPLC method were reported for the quantification of rosuvastin calcium in human plasma by automated solid phase extraction using tandem mass spectrometric detection [2, 3, 4] Its approximate elimination half life is 19 hours and it's time to peak plasma concentration are reached in 3–5 hours following oral administration.Ezetimibe [5] (EZTB), (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxy phenyl)-2-azetidinone, is a class of lipid-lowering compound that selectively inhibits the intestinal absorption of cholesterol and related phytosterols.Several analytical methods have been

developed for the quantification of Ezetimibe .the methods include HPLC[6] and spectrophotometry[7] .Literature survey revealed that no HPTLC method has been reported for the estimation of in combined dosage form.Because of the absence of an official pharmacopoeial method for the simultaneous estimation of ROS and EZE in tablet dosage form, efforts were made to develop an analytical method for the estimation of ROS and EZE in tablet dosage form using HPLC method.

Material and Methods

The instrument used for the estimation, was Camag Linomat V semi automatic sample applicator, Camag TLC scanner 3, CATS software for interpretation of the data, Hamilton syringe and Camag twin trough chamber.ROS and EZE pure powder were procured as gifts sample from Sun pharma Dadra. Rozavel EZ tablets (Sun Pharmaceuticals Ltd) were procured from local market. Label claim of Rozavel EZ tablet for ROS and EZE were 10 mg and 10 mg respectively. n-butanol, Methanol HPLC grade, Chloroform were purchased from E.Merck (Mumbai, India). Working standards of the equivalent of 10 mg each of ROS and EZE were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 µg/ml of ROS and EZE.Twenty tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of ROS and 10 mg EZE was taken in 25ml volumetric flask and dissolved in 75ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through a.whatman no 41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent.The extracts were filtered through Whatman filter paper 41 and required dilutions were made to get the final concentration containing 0.05µg/µl ROS, 0.015 µg/µl EZE, 6 µl ofstandard and sample were applied as 8 mm band on the TLC plate.



Rosuvastatin Calcium **Ezetimibe Molecule**

Results and Discussion

TLC plates were prewashed with methanol and activated prior to use. The chromatographic conditions maintained were: Precoated Silica gel 60 F_{254} (20×10 cm) aluminum sheets as stationary phase. n-butanol: methanol (3:1) as a mobile phase for both the ROS and EZE combinations. Samples were applied as bands 8 mm width at 11.5 mm intervals using Camag

linomate V semiautomatic sample applicator and migration distance allowed was 54 mm, drying of plate done for 12 min at 90^{o} temperatures. The plates were scanned at 274 nm for ROS and EZE and 230 nm for combination with Camag TLC scanner III, using Camag Win CATS software and the source of radiation of deuterium lamp. On to a pre-washed and activated TLC plate, 5-15 ml of standard stock solution of ROS and EZE was spotted with Linomat V Semi applicator. The plates were developed and scanned. The peak areas of each standard were obtained from the system, and a calibration graph was plotted with concentration vs. peak area. The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra - day assay precision, repeatability of measurement, and repeatability of sample application. From the sample aliquot prepared, 3 and 7 ml solution was applied, and the plate was developed with the mobile phase. A triplicate of those was carried out, and the peak areas were noted.

The amount of ROS and EZE present in the formulation was calculated using the respective calibration graph. To develop a precise, accurate and suitable HPTLC method for the quantitative determination of ROS and EZE different solvent systems were employed and the proposed chromatographic condition was found appropriate for the quantitative determination. The mobile phase consisted of chloroform: methanol (6:3:4, v/v) and R_f value of ROS and EZE were found to be 0.12 and 0.35 respectively. Detection was carried out at 274, 230 ROS and EZE respectively. The proposed method has been validated for assay of ROS and EZE in bulk and tablet dosage forms using following parameters [8], [9]. The target analyte concentration of all the two drugs was fixed as 30 µg/ml. linear calibration plots were obtained over the calibration ranges tested, i.e., 200 to 400 ng/spot, 300 to 600 ng/spot ROS and EZE, respectively. The corresponding linear regression equations, with correlation coefficient ≥0.001, were y=0.3619x+2.9843.02; y=2.3021 x+ 0.9483.21, ROS and EZE, respectively. Accuracy of the method was checked by recovery study using standard addition method, [Table-2] known amount of standard ROS and EZE were added into pre analyzed samples separately and subjected them to the proposed HPTLC method. These studies were carried out at three levels i.e., multiple level recovery studies. The intra- and inter-day precision were carried out at three different concentration levels, i.e., 100,300,500 ng/spot; 200, 400, 600 ng/spot for the determinations of ROS and EZE, respectively. The low values of percentage relative standard deviation (% RSD) for intra-and inter-day variation as shown in [Table-3] reveal that the proposed method is precise. For calibration curve, 0.2, 0.4, 0.6, 0.9 and 1.0 µg/µl standard solution of ROS and EZE were applied on TLC plate. The TLC plates were dried, developed and analyzed as described earlier. Filtered solutions (8 µl) of the marketed formulations were spotted on to the plate followed by development scanning. The analysis was repeated six times, the spot was resolved into two peaks in the chromatogram of drug samples. The contents were calculated from the peak areas of standards and samples recorded. A solvent system that would give dense and compact spots with appropriate and significantly different R_f values was desired for quantification of ROS and EZE combinations. The mobile phase consisting of n-Butanol : methanol (3:1 V/V) R_f value of 0.22±0.01, 0.34±0.01, respectively .The developed method was validated in terms of linearity and range, limit of detection, limit of quantification, recovery study, inter days study, intra day study and study by different analysts. The limit of detection for ROS and EZE was found to be 65.1 ng/spot, 54.1 ng/spot, respectively. The assay value for the marketed formulation was found to be within the limits as listed in. The low RSD value indicates suitability of the method for routine analysis of ROS and EZE in pharmaceutical dosage form.

Recovery studies were carried out to study accuracy and precision of the method. These studies were carried out at three levels i.e. multiple level recovery studies. To the powder formulations the pure standard drug were added at 80%, 100% and 120% levels, dilutions were made and analyzed by the method, the % recovery was calculated by using formula, % recovery = (T-A)/S × 100 where, T is total amount of the drug estimated, A is the amount of drug contributed by tablet powder and S is the amount of pure drug added. The results of recovery studies for both the combinations were found to be around 99-105%, indicating that the method is free from interference from excipients. The ruggedness of the method was evaluated by studying analyst to analyst, intra day and inter days variations and the % RSD was calculated, that was found to be within range. From the above results it can be concluded that the HPTLC method is accurate, precise, specific and reproducible and can be used for routine analysis in solid dosage form.

Table 1 Regression Analysis of Calibration Graph for ROS and EZE

Parameter	ROS	EZE
R_f (SD)	0.24	0.87
Linearity and range (ng\spot)	200	600
Linearity detection (ng\spot)	108	132
Limit of quantification (ng\spot)	274	230
Repeatability of application(%RSD)	0,02	0.11
Repeatability of measurement (%RSD)	0.41	0.69
Intraday (%RSD)	0.13	0.19
Inter day (%RSD)	0.24	0.38
LOD[a]	45.32	55.04
LOQ[b]	78.32	82.43

[$] SD = Standard Deviation

Table 2 -Recovery Studies

ROS				EZE			
Label claimed	%Amount added	Found in(µg/ml)	%recovery	Label claimed	%Amount added	Found in(µg/ml)	%recovery
10	80	9.98	98.93	10	80	10.03	100.05
	100	10.02	100.02		100	10.01	100.02
	120	10.06	100.05		120	9.97	99.93

Method Validation

Linearity

Calibration graphs were constructed by plotting peak area Vs concentration of ROS and EZE and the regression equation were calculated.

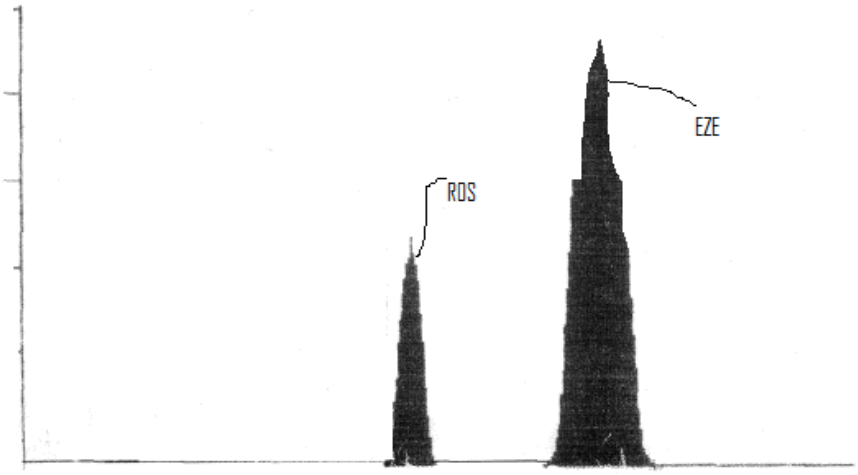
Accuracy

The accuracy of the method was established using recovery technique i.e external standard addition method. The known amount of standard was added at three different levels to

preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in table 2.

Table 3 Result of Assay of Tablet Formulation

ROS		EZE	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
10	9.99	10	9.99
	10.03		9.95
	9.99		10.07
	9.97		9.92
	10.02		9.85
	10.04		10.01
Mean	3.692	Mean	2.904
±SD	0.0381	±SD	0.0431



HPTLC Chromatogram ROS and EZE

Fig. : 1

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of ROS and EZE.

Intermediate precision (reproducibility)
The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of ROS and EZE at concentration three times on the same day and on 3 different days. The results are reported in terms of relative standard deviation.

Limit of detection (LOD) and limit of quantitation (LOQ)
These were studied to determine the sensitivity of the developed method. LOD was calculated using formula, LOD= 3.3×σ/S, where, σ is residual standard deviation of regression line and S is slope of corresponding line. The LOD and LOQ were found to be 220.12 ng and 334.12 ng of the drug, respectively. To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level, to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed and the results obtained are shown in [Table 2]. The results of recovery studies were within the specified limits of ICH guidelines. Lower values of % RSD reflect the accuracy of the method. Precision, expressed in terms of % RSD, was determined in terms of intra-day and Inter-day precisions, analyzing the drug at three different concentrations, determining each concentration thrice. The sample solutions were analyzed using the method for 3 consecutive days, repeating the process twice-a-day at different period. The results obtained are summarized in reflect high degree of precision.

Conclusion

Two different analyst performed assay on marketed tablets of the drug**,** in similar operational and environmental conditions, using the developed method to determine its ruggedness, and the results are summarized. The optimized solvent system yielded a symmetrical peak for the drug with R_f 0.303. A typical absorbance spectrum of the drug is shown in figure 1. The peak for the drug from tablets was identified by comparing the $R_{f,}$ and also comparing its absorbance spectrum with that obtained with the standard drug. The proposed method has advantage of simplicity and convenience for the separation and quantitation of ROS and EZE in the combination and can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for simultaneous estimation of Rosuvastatin Calcium and Ezetimibe in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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