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Research Article

**SIMULTANEOUS ESTIMATION OF ROSUVASTATIN
CALCIUM AND EZETIMIBE AS BULK DRUG AND IN TABLET
DOSAGE FORM BY RP-HPLC METHOD****Padmanabh Deshpande*and Amit Gunge**All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Quality
Assurance Techniques, Kennedy Road, Near RTO, Pune-411001**Abstract:**

A simple, rapid, specific and sensitive reverse phase high performance liquid chromatography was developed and validated for simultaneous estimation of Rosuvastatin calcium and Ezetimibe in combined tablet formulation. The separation was achieved by Thermo C18 column (4.6mm x 250 mm) with a mobile phase consisting of Acetonitrile: Water (80: 20 v/v) at a flow rate of 1.0 mL min⁻¹. Detection wavelength was 241 nm. Retention times were found to be 1.758 min and 3.133 min for Rosuvastatin calcium and Ezetimibe, respectively. The developed method was validated interms of linearity, precision, accuracy, limit of detection, limit of quantitation and robustness. The method was found to be linear in the concentration range 5-30 µg mL⁻¹ for both the drugs. The method was applied successfully for the analysis of drugs in combined tablet dosage form. The mean % recovery was found to be 101.16 and 100.11 for Rosuvastatin calcium and Ezetimibe, respectively. The developed method can be used for the simultaneous quantification of these drugs in the dosage form, bulk drug as well as for routine analysis in quality control laboratories.

Keywords: *Rosuvastatin, Ezetimibe, RP-HPLC; Method validation, ICH Guidelines.***Corresponding author:****Dr. Padmanabh B. Deshpande,**

Assistant Professor

Department of Quality Assurance,

AISSMS College of Pharmacy

Kennedy Road, Near R.T.O.

Pune-411001, Maharashtra, India

Ph. No. +91-9763740388

E-mail: padmanabh77@yahoo.co.in

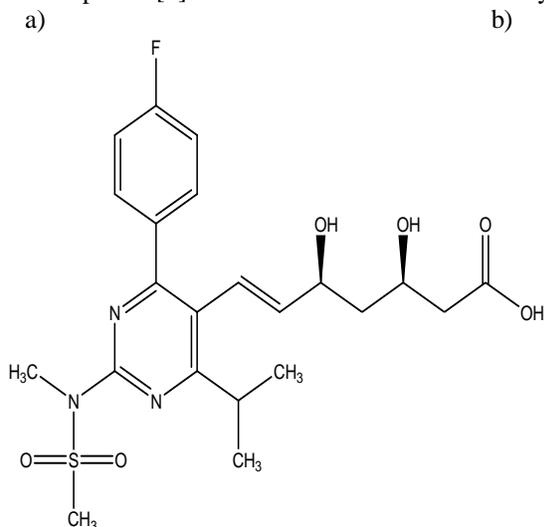
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INTRODUCTION:

Rosuvastatin calcium (RSV), chemically is Bis-[(E)-7 [4-(4-fluorophenyl)-6 isopropyl-2- [methyl (methyl sulphonyl) amino] pyrimidin-5-yl] (3R, 5S) -3,5-dihydroxyhept-6-enoic acid], calcium salt [Figure 1(a)] is well-known member of the drug class known as statins, which are used primarily as a lipid-lowering agent that inhibits HMG-CoA reductase enzyme which is found in liver tissue for production of cholesterol [1]. It is official in Indian Pharmacopeia [2]. Ezetimibe (EZT) is 1-(4-fluorophenyl) -(3S)-hydroxypropyl] -(4S) -(4-hydroxyphenyl)-2 -azeti-dinone [Figure 1(b)] is a selective cholesterol absorption inhibitor, which potentially inhibits the absorption of biliary and dietary cholesterol [3]. It is official in Indian Pharmacopeia [4]. Extensive literature survey



revealed that methods such as spectrophotometry [5-7], High performance liquid chromatography (HPLC) [8-12] have been reported for the determination of RSV in pharmaceutical formulations either as single drug or in combination with other drugs. Analytical methods reported for determination of EZT includes UV spectrophotometry [13-14] HPLC [15-16] and HPTLC [17] methods. Simultaneous quantitative determination of RSV and EZT in combination by UV Spectrophotometry [18]. To the best of our knowledge, no reports were found for simultaneous quantification of these drugs by HPLC method in tablet dosage form. This paper describes development and validation of simple, precise, accurate RP-HPLC method for simultaneous estimation of RSV and EZT in accordance with International Conference on Harmonisation Guidelines [19].

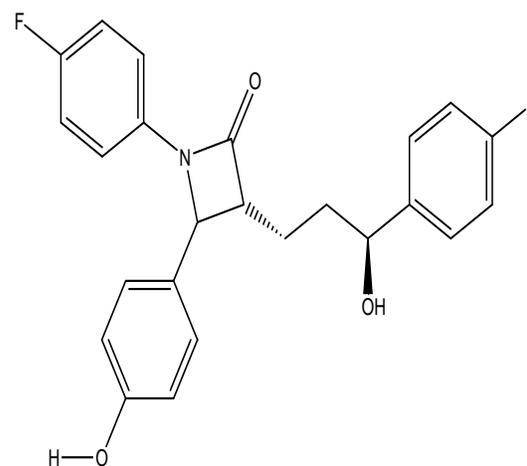


Fig. 1: Chemical structure of a) RSV b) EZT

MATERIAL AND METHODS:**Chemicals and Reagents:**

Analytically pure drug samples of RSV and EZT were received as gift samples from Ajanta Research Centre, Aurangabad. The drug samples were used without further purification. HPLC grade water was generated by double distillation through ELGA LAB WATER purification system (PURELAB UHQ-11, United Kingdom). Acetonitrile (HPLC grade) was purchased from LOBA Chemie, Mumbai (MH, India). Pharmaceutical dosage form Razel EZ tablets manufactured by Glenmark Pharmaceuticals Ltd. labeled to contain 10 mg RSV and 10 mg EZT 10 mg were procured from local pharmacy.

Instrumentation and Chromatographic conditions:

HPLC system used was JASCO system equipped with model PU 2080 Plus pump, Rheodyne sample injection port (50 μ l), JASCO PDA MD-2010 Plus detector and Borwin chromatography software (version 1.5) with HiQSil C18 (250mm*4.6 mm, 5 μ m) column. The optimized mobile phase is Acetonitrile and Water in ratio (80:20 % v/v). The overall run time was 10 min and the flow rate was 1.0 mL min⁻¹. The quantification was carried out at 241 nm. The representative chromatogram of mixed standard solution is represented in Fig. 2.

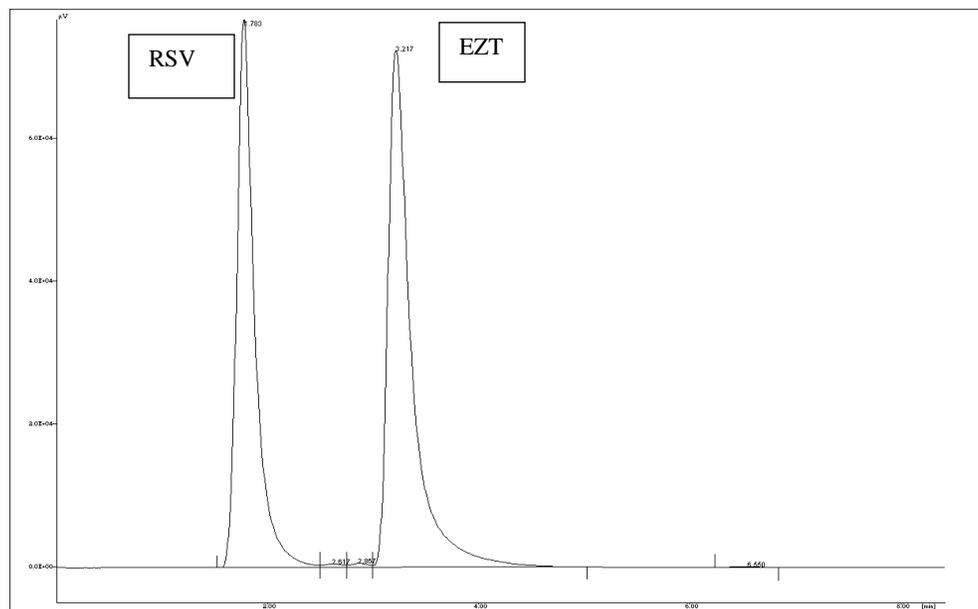


Fig 2: Chromatogram of mixed standard solution of RSV ($10 \mu\text{g mL}^{-1}$, RT = 1.71 min) and EZT ($10 \mu\text{g mL}^{-1}$, RT = 3.25 min)

Preparation of Standard Stock Solution:

Stock solution of RSV and EZT was prepared by dissolving accurately weighed 10 mg of each drug in 10 mL of HPLC grade methanol separately to obtain the solutions having concentration $1000 \mu\text{g mL}^{-1}$ for both drugs. From above solution, further 5 mL of solution was pipette out and diluted to 50 mL to produce $100 \mu\text{g mL}^{-1}$ each of RSV and EZT.

Analysis of Tablet Formulation:

Twenty Tablets each containing 10 mg each of RSV and EZT were weighed and finely powdered. A quantity of powder equivalent to 10 mg of RSV was weighed and transferred to 10 mL volumetric flask containing 7 mL methanol. The solution was sonicated for 10 min and volume was made up to 10 mL with methanol to get concentration of $1000 \mu\text{g mL}^{-1}$ for both drugs. Appropriate dilutions were made with methanol to furnish the final concentration $10 \mu\text{g mL}^{-1}$ and then it is injected. The procedure was repeated for six times. The results obtained for assay of RSV and EZT are shown in Table 1.

Method Validation:

The method was validated with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines [19].

Linearity:

Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of working standard solution of both drugs solution ($100 \mu\text{g mL}^{-1}$ each of RSV and EZT) were transferred into a series of 10 mL volumetric flasks, and the volume was made up to the mark with the mobile phase. Five

replicates per concentration were injected and chromatograms were recorded. The peak areas were recorded and a calibration curve of peak area against concentration of drug was plotted.

Precision:

A set of three different concentrations of mixed standard solutions of RSV and EZT were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. For interday variation study, three different concentrations of the mixed standard solutions in the linearity range were analyzed on three consecutive days. The peak areas were recorded and the relative standard deviation (RSD) was calculated for both series of analyses.

Accuracy:

To check the accuracy of the method, recovery studies were carried out by the addition of standard drug solution to a preanalyzed sample solution at three different levels, 50%, 100%, and 150%.

LOD and LOQ:

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as $3.3 \sigma/S$ and $10 \sigma/S$, respectively, where σ is the SD of the response (yintercept) and S is the slope of the calibration plot.

Robustness:

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase ($0.8 \pm 0.02 \text{ mL min}^{-1}$),

a wavelength at which the drugs were recorded (241 ± 1 nm), and mobile phase percentage with respect to acetonitrile ($\pm 2\%$). One factor was changed at a time to estimate the effect. The solutions containing $10 \mu\text{g mL}^{-1}$ of RSV and $10 \mu\text{g mL}^{-1}$ of EZT were applied onto the column. A number of replicate analyses ($n = 3$) were conducted at three levels of the factor ($-$, 0 , $+$). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION:

Results were found to be linear in the concentration range of $5\text{-}30 \mu\text{g mL}^{-1}$ for both drugs. The correlation coefficients for the calibration plots were 0.999 for RSV and 0.999 for EZT. The linear regression equations of calibration curve were found to be $y = 75254x + 23939$ for RSV and $y = 95718x - 4289$ for EZT. The calibration curves obtained for RSV and EZT are shown in Fig. 3.

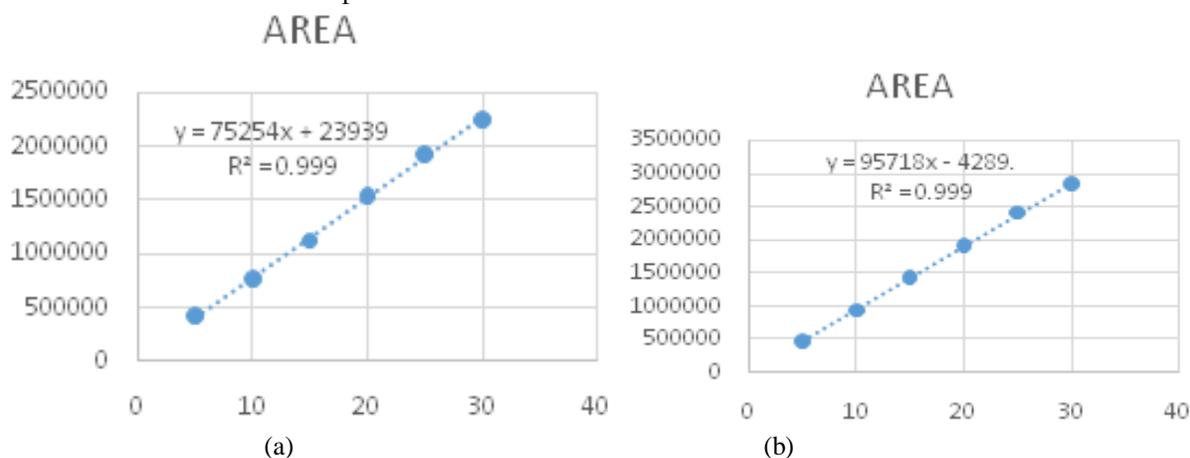


Fig. 3: Calibration curve for (a) RSV and (b) EZT

The proposed method was also evaluated by the assay of commercially available tablets containing RSV and EZT. The % assay was found to be 101.16 ± 0.62 for RSV and 100.11 ± 0.54 for EZT (mean \pm SD, $n = 6$). Intra-day variation, as RSD (%), was found to be in the range of 0.30- 1.50 for RSV and 0.50-1.60 for EZT. Interday variation, as RSD (%) was found to be in the range of 0.48-1.11 for RSV and 0.63-1.27 for EZT. The lower values of %

R.S.D. (< 2) indicated that method was found to be precise.

The average recovery (mean \pm SD) by standard addition method was found to be 100.29 ± 1.17 for RSV and 100.77 ± 1.07 for EZT. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. The results of recovery studies are represented in Table 1.

Table 1: Recovery Studies of RSV and EZT

Drug	Injected concentration ($\mu\text{g mL}^{-1}$)	Added concentration ($\mu\text{g mL}^{-1}$)	Total amount found ($\mu\text{g mL}^{-1}$)	% Recovery	% RSD*
RSV	10	5	15.03	100.20	1.05
	10	10	19.97	99.85	1.78
	10	15	25.21	100.84	0.68
EZT	10	5	15.17	101.13	1.68
	10	10	19.98	99.99	0.89
	10	15	25.30	101.20	0.65

*Average of three determinations

LOD values were found to be $0.224 \mu\text{g mL}^{-1}$ and $0.068 \mu\text{g mL}^{-1}$ for RSV and EZT whereas LOQ values were found to be $0.691 \mu\text{g mL}^{-1}$ and $0.209 \mu\text{g mL}^{-1}$ for RSV and EZT, respectively. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters, showed no

marked changes in the chromatograms ($\text{RSD} < 2$), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed RP-HPLC method is represented in Table 2.

Table 2: Summary of validation parameters of proposed RP-HPLC method

Validation parameters	RSV	EZT
Linearity ($\mu\text{g mL}^{-1}$)	5-30	5-30
Intra-day precision (% R.S.D.)	0.30- 1.50	0.50-1.60
Inter-day precision (% R.S.D.)	0.48-1.11	0.63-1.27
LOD ($\mu\text{g mL}^{-1}$)	0.224	0.068
LOQ ($\mu\text{g mL}^{-1}$)	0.691	0.209
Assay (Mean \pm %R.S.D.)	101.16 \pm 0.62	100.11 \pm 0.54
Accuracy (Mean % recovery)	100.29 \pm 1.17	100.77 \pm 1.07
Robustness	Robust	Robust
Specificity	Specific	Specific

CONCLUSION:

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise, and robust; thus, it can be used for routine analysis of RSV and EZT in combined tablet dosage form.

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