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## ESTIMATION OF SOLID DOSAGE FORMS OF COMBINED ANTIHYPERLIPIDAEMIC DRUGS BY VALIDATED REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD

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### Keywords:

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**ABSTRACT:** A reverse-phase high-performance liquid chromatographic method (RP-HPLC) was developed for the simultaneous estimation of combined dosage form of ezetimibe with simvastatin and rosuvastatin using RP-C18 column. For estimation of ezetimibe and simvastatin, the mobile phase (acetonitrile: water and pH adjusted to 4.5 with acetic acid) was pumped at a flow rate of 0.8 ml/min in the ratio of 85:15% v/v and the eluents were monitored at 234 nm. For the estimation of ezetimibe and rosuvastatin the mobile phase (acetonitrile: water and pH adjusted to 4.5 with acetic acid) was pumped at a flow rate of 0.8 ml/min in the ratio of 75:25% v/v and the eluents ezetimibe and rosuvastatin were monitored at 245 nm. Linearity was obtained in the concentration range of 2-12 µg/ml for ezetimibe in both the mobile phase, 2-12 µg/ml for simvastatin, and 2-12 µg/ml for rosuvastatin. The method was statistically validated, and RSD was found to be less than 2% indicating high degree of accuracy and precision of the proposed method. Due to its simplicity, rapidness, high precision, and accuracy, the proposed RP-HPLC method can be applied for simultaneous determination of ezetimibe and its combined drug simvastatin and rosuvastatin from the bulk and formulation.

**INTRODUCTION:** Ezetimibe (EZE) chemically (3R, 4S)-1-(4-fluorophenyl) -3-((3s)-3-(4-fluorophenyl)-3-hydroxypropyl)-4-(4-hydroxyphenyl) -2-azetidinone is an antihyperlipidaemic agent that has usefulness in lowering cholesterol levels. It acts by decreasing cholesterol absorption in the intestine by blocking the absorption of the sterol at the brush border.

Specifically, the β-lactam binds to the Niemann-pick C1 like 1(NPC1L1) protein on the gastrointestinal tract that is responsible for cholesterol absorption. Although it may be used alone, it is marketed as a combination product with simvastatin <sup>1-4</sup>.

Various analytical methods have been reported for the estimation of EZE alone or in combination with other antihypertensive agents or anti-hyperlipidemic drugs in pharmaceutical formulations; these include reverse-phase high-performance liquid chromatography <sup>5-7</sup>, degradation study <sup>8</sup>, UV spectroscopy <sup>9-10</sup>, bioanalytical LC-MS/MS method <sup>11-12</sup>. Simvastatin (SIM) chemically 2-2-dimethyl butanoic acid, 1, 2,

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3, 7, 8, 8a-hexahydro, 3, 7- dimethyl- 8-[2(tetrahydro-4hydroxy-6-oxo-2pyran-2-yl) ethyl]-1- naphthalenyl ester is an analog of lovastatin, in liver undergo extensive metabolism to several opening hydroxyl acids including the active  $\beta$ -hydroxy acids. They are also highly bound to plasma proteins<sup>1-4</sup>.

Analytical methods have been reported for the estimation of SIM alone or in combination with other antihypertensive agents or anti-hyperlipidemic drugs in pharmaceutical formulations, these include reverse-phase high-performance liquid chromatography<sup>13-15</sup>, LC-MS/MS method<sup>16</sup>, solubility study method<sup>17</sup>, bioanalytical method<sup>18-19</sup>, polarographic method<sup>20</sup>. Rosuvastatin (RV) chemically 7- [4- (4-

fluorophenyl) - 6- (1-methylethyl)- 2- (methyl-methylsulfonyl - amino)-pyrimidin-5-yl] 3, 5-dihydroxy-hept-6-enoic acid is one of the more recently introduced statins. Statins are HMG-CoA reductase inhibitors. The conversion of 3-hydroxy 3 methyl glutaryl (HMG)-COA to mevalonic acid is especially important because it is a primary control site for cholesterol biosynthesis<sup>1-4</sup>.

Methods such as RP-HPLC<sup>21-25</sup>, synchronous spectrophotofluorometry<sup>26</sup>, UV spectrophotometric methods<sup>27</sup>, HS GC MS<sup>28</sup> have been reported for estimation of RV alone or in combination with other drugs. The drug simvastatin is official in BP<sup>29</sup>. Ezetimibe, simvastatin, and rosuvastatin are official in IP<sup>30</sup>. The chemical structures of EZE, SIM, and RV are shown in Fig. 1.

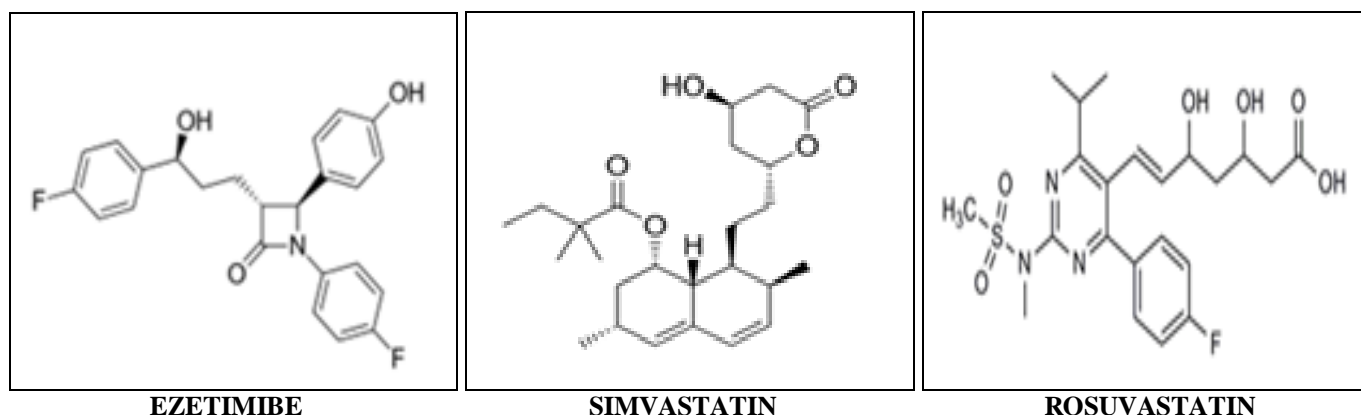


FIG. 1: CHEMICAL STRUCTURES OF DRUGS

A literature survey revealed that several methods have been reported for the estimation of EZE, SIM, and RV individually as well as in combination with some other antihypertensive drugs. However, in analysis, good approach is to develop a specific method that avoids interference of other drugs in determination from their combined mixture. In present piece of work, an attempt has been made to develop a suitable analytical method for simultaneous estimation of EZE and SIM, EZE, and RV in formulation. HPLC methods have been widely used for routine quality control assessment of drugs because of their accuracy, repeatability, selectivity, sensitivity and specificity. Analytical methods must be validated before use the proposed HPLC-UV detection method was validated in accordance with International Conference on Harmonization (ICH) guidelines<sup>31, 32</sup> by assessing its selectivity, linearity, accuracy, and precision, limit of detection and limit of quantitation.

## MATERIALS AND METHODS:

**Instrumentation:** Analysis was performed with a Shimadzu (Japan) prominence chromatograph equipped with an LC - 20 AT solvent delivery system, a universal loop injector (Rheodyne 7725) of injection capacity of 20  $\mu$ l, and an SPD - 20 A UV-Visible detector set at 234 nm for EZE and SIM, at 245 nm for RV and EZE. The equipment was controlled by a PC work station with clarity software. Compounds were separated on the Phenomenex Luna C<sub>18</sub> column (250 mm  $\times$  4.6 mm.i.d., 5- $\mu$ m particle size) under reversed-phase partition conditions. For estimation of EZE and SIM the mobile phase was an 85:15% (v/v) mixture of Acetonitrile: Water (pH 4.5  $\pm$  0.2, adjusted with Acetic acid). For estimation of EZE and RV the mobile phase was a 75:25% (v/v) mixture of Acetonitrile: Water (pH 4.5  $\pm$  0.2, adjusted with Acetic acid). The flow rate was kept at 0.8 ml/min, and the run time was 8 min.

Before analysis both the mobile phase and sample solutions were degassed by the use of a sonicator (Lab man scientific Instruments Chennai) and filtered through a 0.22  $\mu\text{m}$  filter (Pall Corporation, Mumbai). The identity of the compounds was established by comparing the retention times of compounds in the mixed solution with those in standard solutions. Chromatography was performed in an ambient temperature maintained at  $40 \pm 1$  °C. The UV spectrum of EZE, SIM, and RV for selecting the working wavelength of detection was taken using a Shimadzu-1700 a double beam UV - Visible spectrophotometer (Shimadzu, Kyoto, Japan).

**Reagents and Chemicals:** Pharmaceutically pure samples of EZE & SIM were procured from Swapnroop Pharma Aurangabad and RV from Ajanta pharma Aurangabad as a gift sample. HPLC grade acetonitrile, water was obtained from Merck life sciences Pvt. Ltd. and acetic acid (HPLC grade) were obtained from Qualigens India Pvt. Limited, Mumbai, India. Formulations Simcard-EZ contains SIM 10 mg and EZE 10 mg manufactured by Cipla Ltd and Rozavel EZ contains RV 10 mg and EZE 10 mg manufactured by Sun Pharma was purchased from local market.

**Preparation of Standard Solutions of EZE, SIM, and RV:** About 10 mg of each drug was accurately weighed separately and transferred to separate 10 ml volumetric flasks. The pure drugs were dissolved in mobile phase, and the volume was made up to the mark, *i.e.* 10 ml with mobile phase. From this standard stock solution, the aliquots of solution were further diluted with mobile phase to obtain standard solutions with conc. range 1 – 12  $\mu\text{g/ml}$  for EZE, 2 - 12  $\mu\text{g/ml}$  for SIM and 2-12  $\mu\text{g/ml}$  for RV. The mixed standard solution was also prepared simulated to marketed formulations.

**Plot of Calibration Curve:** Calibration curve for each drug was plotted at the concentrations of 2-12  $\mu\text{g/ml}$  for EZE, 2-12  $\mu\text{g/ml}$  for SIM and 2-12  $\mu\text{g/ml}$  for RV. All the standard solutions were filtered through a syringe filter, degassed for 10 min in the sonicator and injected 20  $\mu\text{l}$  solutions in the column by Hamilton syringe. The obtained chromatograph was read and the peak areas were measured. Peak areas were then plotted against their respective concentrations for EZE, SIM, and RV.

Standard regression curve analysis was obtained by the use of Microsoft Office Excel software and Means, standard deviations and coefficient of variance were calculated.

**Assay of Tablet Formulation:** 20 tablets of formulation containing 10 mg SIM and 10 mg EZE were weighed and triturated to powder, powder equivalent to 5 mg of EZE and 5 mg of SIM was accurately weighed and transferred to a 10 ml volumetric flask and dissolved into mobile phase and volume was made up to 10 ml with mobile phase. The solution was sonicated for 15 min, to ensure the uniform and homogenous solution of the drugs. The solution was filtered through a membrane filter (pore size 0.45 $\mu$ ). The aliquot of solution was further diluted to 5 ml into clean and dry volumetric flask with mobile phase to obtain conc. of 2  $\mu\text{g/ml}$  of EZE and 2  $\mu\text{g/ml}$  of SIM.

Similarly 20 tablets of formulation containing 10 mg of EZE and 10 mg of RV were weighed and triturated to powder, powder equivalent to 5 mg of EZE and 5 mg of RV was weighed and transferred to a 10 ml volumetric flask and dissolved into mobile phase and volume was made up to 10 ml with mobile phase. The solution was sonicated for 15 min, to ensure the uniform and homogenous solution of the drugs. The solution was filtered through a membrane filter (pore size 0.45 $\mu$ ). The aliquot of solution was further diluted to 5 ml into clean and dry volumetric flask with mobile phase to obtain conc. of 2  $\mu\text{g/ml}$  of EZE and 2  $\mu\text{g/ml}$  of RV. Before injecting the formulation solutions were filtered through syringe filter, degassed for 10 min in the sonicator, and injected 20  $\mu\text{l}$  solutions in the column by Hamilton syringe. The obtained chromatograph was read and the peak areas were measured to know the conc. of tablet solution.

**Validation:** The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, selectivity, and specificity. Accuracy was assessed by measuring recovery at three different levels. Precision assessed by measurement of intra and inter-day precision. Selectivity and specificity of the method were assessed by injecting solutions containing the drugs.

After chromatography two sharp peaks were obtained for EZE and SIM, RV and EZE. LOD and LOQ were measured to evaluate the detection and quantitation limits of the method and to determine whether these were affected by the presence of impurities. These were calculated by using equations,  $LOD = 3.3 \sigma/S$  and  $LOQ = 10 \sigma/S$ , where  $\sigma$  is the standard deviation of the response and S is the slope of the calibration plot.

## RESULTS AND DISCUSSION:

### HPLC Method Development and Optimization:

The multi-component formulations have gained a lot of importance as there is greater patient acceptability, increased potency and decreased side effects. EZE, SIM, and RV are used for the treatment of hypertension, as anti-hyperlipidemic. This work was focused on optimization of the conditions for the simple and rapid as well as low cost and effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results.

To optimize mobile phase, the chromatogram of all drugs were obtained in mobile phase acetonitrile: water in the ratio 70:30% v/v, all three drugs eluted but retention time of simvastatin was more than 12. Therefore, the proportion of acetonitrile was

increased to 80% that resulted in elution of all drugs within 10 min. mobile phase composition was changed to methanol: water in proportion 80:20% v/v, the result was improper resolution. Therefore, mobile phase of acetonitrile: water in composition 75:25% v/v (pH 4.5 adjusted with acetic acid) was selected for RV and EZE, mobile phase of acetonitrile: water in composition 85:15% v/v (pH 4.5 adjusted with acetic acid) was selected for SIM and EZE. These changes resulted in elution of drugs with reasonable retention factors and symmetry in peak. To determine the appropriate wavelength for simultaneous determination of EZE and SIM, EZE and RV solutions of these compounds in mobile phase were scanned in the range of 200 - 400 nm.

From the overlaid UV spectra, suitable wavelength considered for monitoring the drugs was 245 nm (For RV and EZE) and 234 nm (For SIM and EZE). It was observed that analytes absorbed well at 245 nm and 234 nm, and at this wavelength, there was no interference from the mobile phase or baseline disturbance, and it was, therefore, concluded that 245 nm and 234 nm was the most appropriate wavelength for analysis of drugs with suitable sensitivity.

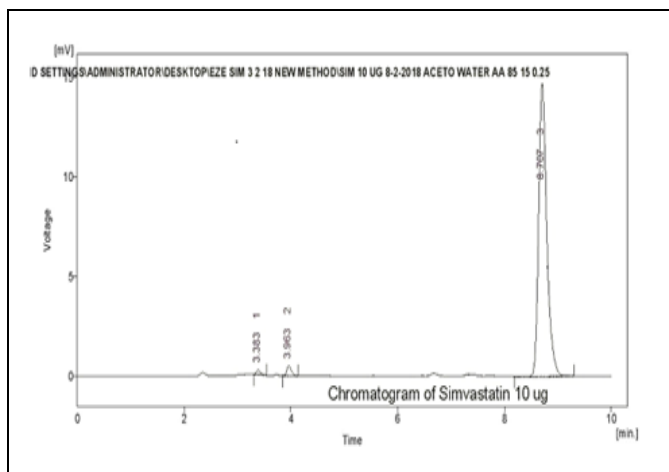


FIG. 2: OPTIMIZED CHROMATOGRAPH OF SIMVASTATIN

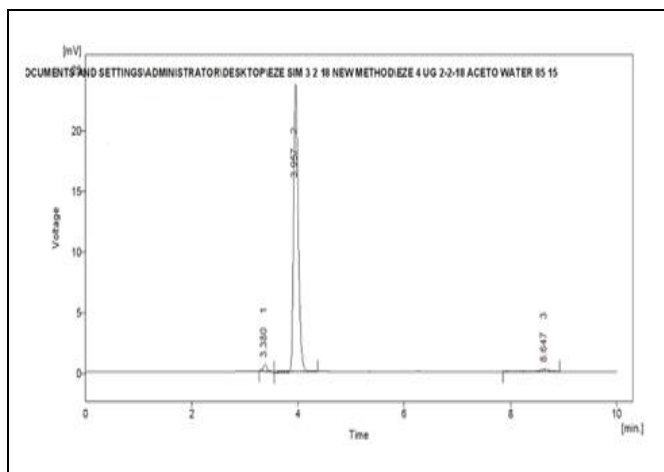
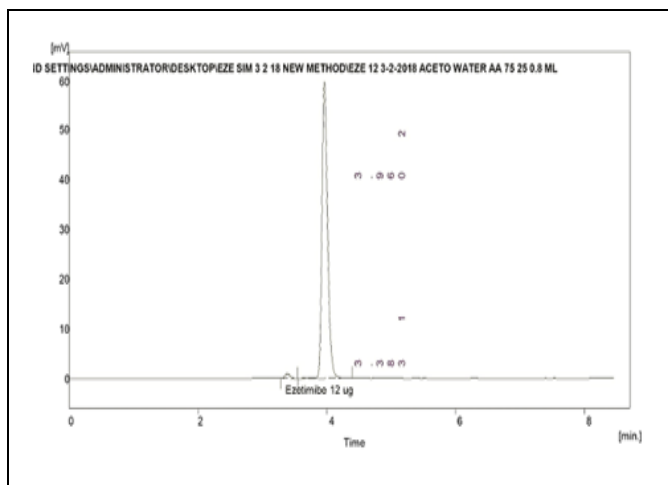


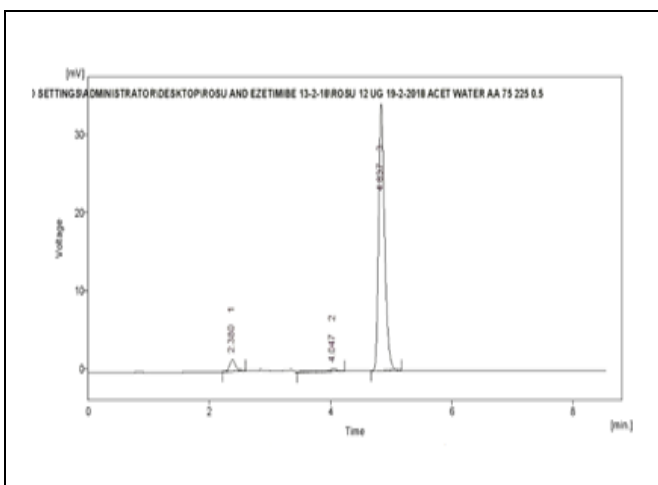
FIG. 3: OPTIMIZED CHROMATOGRAPH OF EZETIMIBE

The optimum mobile phase was, therefore, Acetonitrile: water (pH 4.5  $\pm$  0.1) in the ratio of 85:15% (v/v), under these experimental conditions, sharp peaks of SIM and EZE obtained were shown in Fig. 2 and 3 respectively and with the retention time 3.957 and 8.697 min. respectively. Similarly, chromatographic peaks of EZE and RV obtained

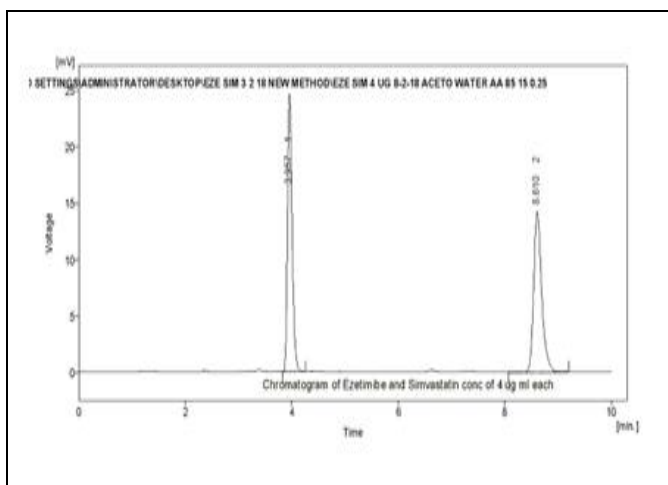
were shown in Fig. 4 and 5 respectively) and with the retention time 4.013 and 4.697 min. respectively. The resolution (RS) between SIM and EZE was 2.49 and Between RV and EZE 5.694. Chromatographic conditions were unaffected by varying conc. of drugs in mixture and shown in chromatogram Fig. 6 and 7.



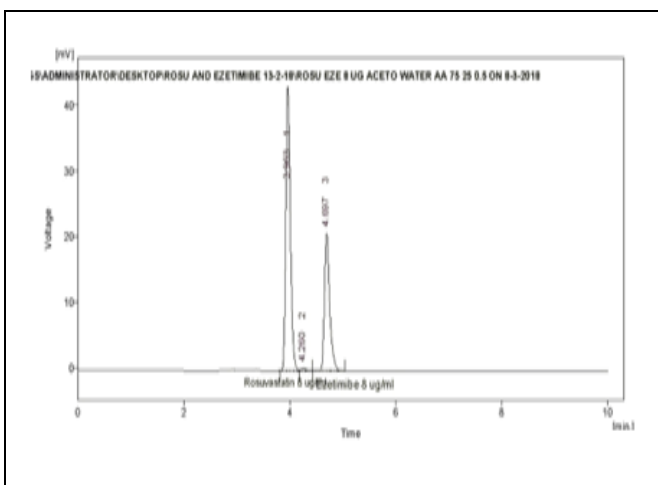
**FIG. 4: OPTIMIZED CHROMATOGRAPH OF EZETIMIBE**



**FIG. 5: OPTIMIZED CHROMATOGRAPH ROSUVASTATIN**



**FIG. 6: CHROMATOGRAPH OF EZETIMIBE AND SIMVASTATIN**



**FIG. 6: CHROMATOGRAPH OF EZETIMIBE AND ROSUVASTATIN**

**Method Validation:** The system suitability parameters like capacity factor, number of theoretical plates, and tailing factor for all the analyte were found to be within the limit indicating the suitability of the system **Table 1**. The values obtained for  $k'$  ( $1 < k' < 10$ ) and  $RS (>2)$  are demonstrated that selected chromatographic

conditions are appropriate for separation and quantification of all compounds. The number of theoretical plates and the tailing factor were within the acceptance criteria of  $>2000$  and  $\leq 1.5$ , respectively, indicating good column efficiency and optimum mobile phase composition.

**TABLE 1: RESULTS FROM SYSTEM SUITABILITY STUDY**

Parameters	Method of EZE and SIM		Method of RV and EZE	
	EZE	SIM	EZE	RV
Retention time*	3.957	8.697	4.013	4.697
Tailing factor	1.260	1.285	1.204	1.260
Asymmetrical factor	1.294	1.311	1.320	8.457
Number of Theoretical plates	10780	16980	16057	9708
Resolution	3.971	12.541	3.926	8.457
Flow rate ml/m	0.8 ml/min	0.8 ml/min	0.8 ml/min	0.8 ml/min

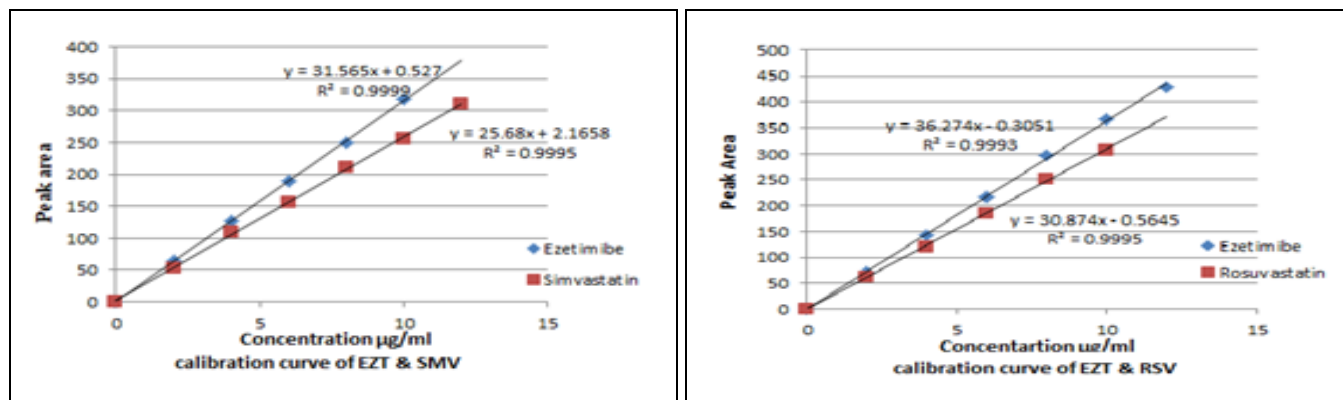
\*Mean of six determinations.

**Linearity:** Linearity was tested in the concentration range 2-12  $\mu\text{g/ml}$  for EZE, 2-12  $\mu\text{g/ml}$  for SIM, and 2-12  $\mu\text{g/ml}$  for RV. To obtain a

calibration curve, standard solutions in the respective conc. range was injected and peak area was measured.

The solutions were chromatographed six times, in accordance with the International Conference on Harmonization. Separate calibration plots for EZE, SIM, and RV were constructed by plotting peak area against the respective concentrations. From the calibration curve, it was found that the drugs were linear in the above concentration range and graphically shown in **Fig. 8**. The calibration graph

was used as a reference to know quantitatively conc. of unknown solution of mixture. The method was evaluated by determination of the correlation coefficient and intercept, calculated in the corresponding statistical study (ANOVA), correlation coefficient  $r^2$  values  $> 0.999$ , and intercept very close to zero confirmed the good linearity of the method **Table 2**.



**FIG. 8: CALIBRATION CURVES**

**TABLE 2: RESULTS FROM STUDY OF LINEARITY**

Parameters	Method of EZE and SIM		Method of EZE and RV	
	EZE	SIM	EZE	RV
Detection wavelength	234 nm	234 nm	245 nm	245 nm
Beer's law limit (µg/ml)	2 - 12	2 - 12	2 - 12	2 - 12
Correlation coefficient (r)	0.9995	0.9994	0.9993	0.9996
Regression equation ( $y = mx + c$ )	$Y = 31.565 X + 0.527$	$Y = 25.68 X + 1.165$	$Y = 36.274 X - 0.3051$	$Y = 30.874 X - 0.5645$
LOD (µg/ml)	0.1964	0.2965	0.1879	0.1555
LOQ (µg/ml)	0.5952	0.8972	0.5696	0.4712

**Assay of Tablet Formulation:** The percentage label claim present in tablet formulation was found to be for 99.73% and 101.28% for SIM and EZE, 99.87% and 101.31% for RV and EZE respectively shown in **Table 3**. The precision of the method was confirmed by the repeated analysis of formulation

for six times. The calculated % COV values were tabulated in **Table 3**. The low % COV values indicated that all the three drugs showed good agreement with the label claim ensures the precision of the method.

**TABLE 3: RESULTS FROM ASSAY OF TABLET FORMULATION**

Formulation	Drug	Label claim (mg/Tablet; n = 6)	Amount found mg	Drug Content %	Std Deviation	Coeff. of variance	RSD
Formulation	EZE	10	10.128	101.28	1.8789	1.8521	0.0231
	SIM	10	9.9730	99.73	2.3041	2.3101	0.0185
Formulation	EZE	10	10.1310	101.31	2.0639	2.0312	0.0203
	RV	10	9.9871	99.87	1.4550	1.4511	0.0145

Std – Standard Coeff - Coefficient

**Precision:** Precision was determined by repeating assay six times and SD and RSD gave in **Table 4**.

**Accuracy:** To check the accuracy of the developed methods and to study the interference of drug components, analytical recovery experiments were carried out as per ICH guidelines. The results of the recovery studies and its statistical validation data

given in **Table 4** indicate high accuracy of the proposed method. The percentage recovery was found to be in the range of 101.23-103.49% for SIM, 98.13-99.73% for EZE, and 98.33-99.91% for RV, 97.99-98.69 for EZE. The % COV values for SIM, EZE formulation and RV, EZE formulation were given in **Table 4**.

**Robustness:** As defined by the ICH, the robustness of an analytical procedure describes its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the

chromatographic conditions and found to be unaffected by small variations like  $\pm 1\%$  variation in volume of mobile phase composition,  $\pm 0.1$  ml/min variation in flow rate of mobile phase,  $\pm 0.1$  variations in pH.

**TABLE 4: RESULTS OF PRECISION OF THE METHOD / RESULTS OF RECOVERY ANALYSIS**

S. no.	Parameter	Drug Name	S.D.	RSD	COV
1	Precision of EZE and SIM	EZE	0.6490	0.00661	0.6617
		SIM	2.0903	0.02061	2.0611
2	Accuracy of EZE and SIM formulation	EZE	0.8467	0.00857	0.8573
		SIM	1.1621	0.01131	1.1301
3	Precision of EZE and RV	EZE	0.8603	0.00869	0.8696
		RV	2.3041	0.02311	2.3101
4	Accuracy of EZE and RV	EZE	0.4509	0.00457	0.0457
		RV	0.9138	0.00932	0.9237

S.D- Standard deviation, COV- Coefficient of variance, RSD Relative standard deviation

**Specificity:** The specificity of the HPLC method was ascertained by analyzing standard drug and sample solutions. The retention time of EZE, SIM, and RV was confirmed by comparing the retention time with that of the standard.

**CONCLUSION:** Drug interference between these anti-hyperlipidemic agents was not observed during this estimation, and a simple isocratic RP - HPLC method with UV detection has been developed for simultaneous determination of EZE and SIM, RV and EZE. The method was validated for accuracy, precision, specificity, and linearity. The run time is relatively short (8 to 10 min), which enables rapid quantification of many samples in routine and quality control analysis of tablets. The method also uses a solvent system with the same composition as the mobile phase for dissolving and extracting drugs from the matrices, thus minimizing noise.

Thus, the proposed method is rapid, selective, requires a simple sample preparation procedure, Moreover, the lower solvent consumption leads to a cost-effective and represents a good procedure of EZE and SIM, RV and EZE determination in bulk and in pharmaceutical dosage forms.

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**CONFLICT OF INTEREST:** The authors declared that they have no conflict of interest.

#### REFERENCES:

1. The Merck Index an Encyclopaedia of chemicals, drugs and Biological. The royal society of chemistry, Cambridge UK, Edition 15<sup>th</sup>. 2013; 720: 1587-40.
2. Beale JM and Jr Block JH: Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical chemistry. Wolters Kluwer (India) pvt. Ltd., New Delhi, Edition 12<sup>th</sup>. 2011: 652-54.
3. Brayfield A: Martindale (The complete drug reference). Pharmaceutical press, London, Edition 38<sup>th</sup>. A, 2014: 1379, 1488, 1489.
4. Lemke TL, Williams DA, Roche VF and Zito SW: Foyes principles of medicinal chemistry. Wolters Kluwer (India) pvt. Ltd., New Delhi, Edition 7<sup>th</sup>. 2013; 826.
5. Raul SK, Aravelli RB and Jhansi D: RP-HPLC method development and validation for the simultaneous estimation of atorvastatin and ezetimibe in the pharmaceutical dosage form. Asian Journal of Pharmaceutical and Clinical Research 2015; 8(2): 178-81.
6. Artici E and Karliga B: Identification, synthesis and characterization of process-related desfluoro impurity of ezetimibe and HPLC method validations. Journal of Pharmaceutical Analysis 2015; 5(6): 356-70.
7. Kumar BP, Vidyadhara S, Murthy TEGK, Rao BV and Nikhila V: A novel reverse phase liquid chromatographic method development and validation for the simultaneous estimation of atorvastatin, ezetimibe and fenofibrate in bulk and tablet dosage form. Int Journal of Pharmaceutical Sciences and Research 2016; 7(10): 4145-51.
8. Santa Z, Koti J, Katalinszoke, Vukics K and Jr Szantay C: Structure of the major degradant of ezetimibe. Journal of Pharma and Biomedical Analysis 2012; 58: 125-29.
9. Abdelwaheb NS, El-Zeiny BA and Tohamy SI: Two spectrophotometric methods for simultaneous determination of some anti-hyperlipidemic drugs. Journal of Pharmaceutical Analysis 2012; 2(4): 279-84.
10. Belal TS, Daabees HG, Abdel-Khalek MM, Mahrous MS and Khamis MM: New simple spectrophotometric method for determination of the binary mixtures (atorvastatin calcium and ezetimibe; candesartan cilexetil and hydrochlorothiazide) in tablets. Journal of Pharmaceutical Analysis 2013; 3(2): 118-26.

11. Karanam LR, Katakam P, Chandu BR, Hwisa NT and Adiki SK: Simultaneous determination of ezetimibe and simvastatin in rat plasma by stable-isotope dilution LC-ESI-MS/MS and its application to a pharmacokinetic study. *Journal of Pharmal Analysis* 2014; 4(4): 286-94.
12. Guo L, Meng-meng W, He M, Fu-rong Q and Jiang J: Simultaneous determination of ezetimibe and its glucuronide metabolite in human plasma by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J of Chromato B* 2015; 986-987: 108-14.
13. Phaneendra D and Nagamalleswari G: Chromatographic and spectrophotometric methods for simultaneous determination simvastatin and sitagliptin in the combined dosage form. *International Journal of Pharmaceutical Sciences and Research* 2015; 6(3): 1170-75.
14. Sahoo NK: Validation of Assay Indicating Method Development of Simvastatin in Bulk and its Tablet Dosage form by RP-HPLC. *Journal of Applied Pharmaceutical Sciences* 2014; 4(1): 117-22.
15. Clementino A and Sonvico F: Development and validation of a RP-HPLC method for the simultaneous detection and quantification of simvastatin's isoforms and coenzyme Q10 in lecithin/chitosan nanoparticles. *Journal of Pharma and Biomedical Analysis* 2018; 155: 33-41.
16. El-Zailik A, Cheung LK, Wang Y, Sherman V and Chow DSL: Simultaneous LC-MS/MS analysis of simvastatin, rosuvastatin and their active metabolites for plasma samples of obese patients underwent gastric bypass surgery. *J of Pharma and Bio Analysis* 2019; 164: 258-67.
17. Mishra R: Spherical crystallization a novel approach for solubility and dissolution enhancement of simvastatin. *Asian J of pharma and Clin Research* 2016; 9(6): 65-72.
18. Lawson G, Cocks E and Tanna S: Bisoprolol, ramipril and simvastatin in dried blood spot samples using LC-HRMS for assessing medication adherence. *Journal of Pharma and Biomedical Analysis* 2013; 81: 99-107.
19. Alakhali KM: Validation method for measuring simvastatin in human plasma by HPLC-UV and its application in study simvastatin stability in plasma and working solution. *Asian Journal of Pharmaceutical and clinical Research* 2014; 7(7): 131-3.
20. Ramadan AA, Mandil H and Ashram N: Differential pulse polarographic behaviour and determination of simvastatin in pure and pharmaceutical dosage forms using dropping mercury electrode. *Research Journal of Pharmacy and Technology* 2018; 11(7): 2888-94.
21. Moussa BA, Hashem HMA, Mahrouse MA and Mahmoud ST: A validated RP-HPLC method for the determination of rosuvastatin in presence of sacubitril/valsartan in rat plasma: Application to *in-vivo* evaluation of OATP-mediated drug interaction potential between rosuvastatin and sacubitril/valsartan. *Microchemical Journal* 2018; 143(12): 31-38.
22. Hemantkumar T, Sri SD, Rao V and Rao KSY: Validated RP HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical formulation. *International Journal of Pharmaceutical Sciences and Research* 2015; 6(7): 2913-17.
23. Nazareth C, Fizarido G and Vaz C: Cleaning validation of a simple and rapid reversed-phase high-performance liquid chromatography method for the simultaneous estimation of aspirin and rosuvastatin. *Asian Journal of Pharmaceutical and Clinical Research* 2019; 12(3): 261-5.
24. Machairas G, Panderi I, Geballa-Koukoula A, Rozou S and Vonaparti A: Development and validation of a hydrophilic interaction liquid chromatography method for the quantitation of impurities in fixed-dose combination tablets containing rosuvastatin and metformin. *Talanta* 2018; 183: 131-41.
25. Kumar SA, Debnath M, Rao JVLNS and Sankar DG: Development and validation of a sensitive RP-HPLC method for simultaneous estimation of rosuvastatin and fenofibrate in tablet dosage form by using PDA detector in gradient mode. *Research Journal Pharm and Tech* 2016; 9(5): 549-54.
26. El-Abasawi NM, Attia KAM, Abo-serie AAM, Morshedy S and Abdel-Fattah A: Simultaneous determination of rosuvastatin and propranolol in their binary mixture by synchronous spectrofluorimetry. *Spectrochimica A cat Part A: Molecular and Bio Spectroscopy* 2018; 198: 322-30.
27. Kakade PN and Saudagar RB: Spectroscopic Determination and Validation of Rosuvastatin Calcium Concentration in Bulk and Dosage form. *Research Journal of Pharmacy and Technology* 2016; 9(7): 761-3.
28. Ismail A and Alahmad Y: Determination of ethanol and n-hexane residues in bulk rosuvastatin and atorvastatin and their dosage forms using HS-GC-MS developed method. *Research Journal of Pharmacy and Technology* 2018; 11(11): 4829-36.
29. British Pharmacopoeia: Medicines and Healthcare products regulatory agency London 2015; 2: 812.
30. Indian Pharmacopoeia. Government of India, ministry of Health and family welfare. The Indian pharmacopoeia commission, Ghaziabad, Edition 7<sup>th</sup>, Vol II, III, 2014; 1727, 2684, 2730.
31. International Conference on Harmonization (ICH). Q2A: Text on Validation of Analytical Procedures: Definitions and Terminology. US FDA Federal Register. 1995; 60.
32. International Conference on Harmonization (ICH). Q2B: Validation of Analytical Procedures: Methodology. US FDA Federal Register 1997; 62.

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