International Journal of Current Trends in Pharmaceutical Research
CODEN (USA): IJCTGM | ISSN: 2321-3760
Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article

Analytical Method Development and Validation for Ezetimibe and Rosuvastatin in API and Combine Pharmaceutical Dosage Forms by RP-HPLC

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Abstract

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Rosuvastatin and Ezetimibe in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Rosuvastatin and Ezetimibe was done by RP-HPLC. The Phosphate buffer was pH 2.5 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20% v/v. A Symmetry C18 (4.6x150mm, 5µm, Make X Terra) column used as stationary phase. The detection was carried out using UV detector at 274 nm. The solutions were chromatographed at a constant flow rate of 0.8ml/min. the linearity range of Rosuvastatin and Ezetimibe were found to be from 25-125µg/ml. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Rosuvastatin and Ezetimibe LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Rosuvastatin and Ezetimibe in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

Keywords: Symmetry C18, Rosuvastatin and Ezetimibe, RP-HPLC.

Article Info

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Article History: Received 12 July 2023, **Accepted** 10 Aug 2023, **Available Online** 11 October 2023

Citation: *Dasari Tejaet al., Analytical Method Development and Validation for Ezetimibe and Rosuvastatin in API and Combine Pharmaceutical Dosage Forms by RP-HPLC.Int. J. Curnt. Tren. Pharm, Res.*, 2023, 11(1): 60-65.

CONTENTS

1. Introduction

Rosuvastatin, also known as the brand name product Crestor, is a lipid-lowering drug that belongs to the statin class of medications, which are used to lower the risk of cardiovascular disease and manage elevated lipid levels by inhibiting the endogenous production of cholesterol in the liver. More specifically, statin medications competitively inhibit the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) Reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid and is the third step in a

sequence of metabolic reactions involved in the production of several compounds involved in lipid metabolism and transport including cholesterol, low-density lipoprotein (LDL) (sometimes referred to as "bad cholesterol"), and very low-density lipoprotein (VLDL). Prescribing of statin medication is considered standard practice following any cardiovascular events and for people with a moderate to high risk of development of CVD, such as those with Type 2 Diabetes. The clear evidence of the benefit of statin use coupled with very minimal side effects or long term effects has resulted in this class becoming one of the most widely prescribed medications in North America.Ezetimibe is a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption.

The discovery and research of this drug began in the early 1990s, after the intravenous administration of radiolabelled ezetimibe in rats revealed that it was being localized within enterocytes of the intestinal villi - this prompted studies investigating the effect of ezetimibeon intestinal cholesterol absorption. Ezetimibe is used as an adjunctive therapy to a healthy diet to lower cholesterol levels in primary hyperlipidemia, mixed hyperlipidemia, homozygous familial hypercholesterolemia (HoFH), and homozygous sitosterolemia (phytosterolemia).Unlike other classes of cholesterol-reducing compounds including statins and bile acid sequestrants, ezetimibe has a distinct mechanism of action involving the sterol transporter Niemann-Pick C1-Like 1 (NPC1L1), and is unique in that it does not affect the absorption of fat-soluble nutrients such as fat-soluble vitamins, triglycerides, or bile acids. In genetically NPC1L1-deficient mice, a 70% reduction in intestinal cholesterol absorption was seen, and these mice were insensitive to ezetimibe treatment-it was determined based on these findings that NPC1L1 plays an essential role in promoting intestinal cholesterol uptake via an ezetimibe-sensitive pathway. By interfering with the intestinal uptake of cholesterol and phytosterols, ezetimibe reduces the delivery of intestinal cholesterol to the liver.

2. Methodology

Instrumentation

TheinstrumentusedwasHPLCWatersmodel No. 2695 separation module.2487 UV detector, Software–EMpower. The stationaryphase used was Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column. Digital weighing balance-ModelnumberAfcoset ER-200A,Sonicator(Enertech)-SE60US, pHmeter ModelnumberAdwa – AD 1020, UV/VIS spectrophotometer LABINDIA UV 3000^{+}.

Materials and reagents

Rosuvastatin and Ezetimibe were gift samples provided by Dr. Reddy`s Laboratories, Hyderabad,HCl, H_2O_2, NaOHwere supplied by Merck India Ltd , Acetonitrile for HPLC was supplied by MolychemKH_2PO_4 was supplied by Finer chemical LTD Water and Methanol for HPLC was supplied by Lichrosolv.

Method development

Six trials were made by changing the mobile phase ratios and solvents Buffer: Methanol P^H 2.5 (30:70 v/v) Buffer: Methanol P^H 2.5 (30:70v/v)Buffer: Methanol P^H 2.5 (60:40 v/v)Phosphate buffer: Acetonitrile P^H 2.5 (20:80 v/v)Phosphate buffer: Methanol P^H 2.5 (55:45 v/v) Phosphate buffer: Methanol P^H 2.5 (65:35 v/v). Finally, the mobile phase optimized was Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/v.

Chromatographic conditions

The Phosphate buffer was pH 2.5 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/v. A Symmetry C18 (4.6x150mm,5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 274 nm.

3. Results and Discussion

System Suitability: The system suitability of the method was checked by injecting five different preparations of the Rosuvastatin and Ezetimibe standard. The parameters of system suitability were checked.

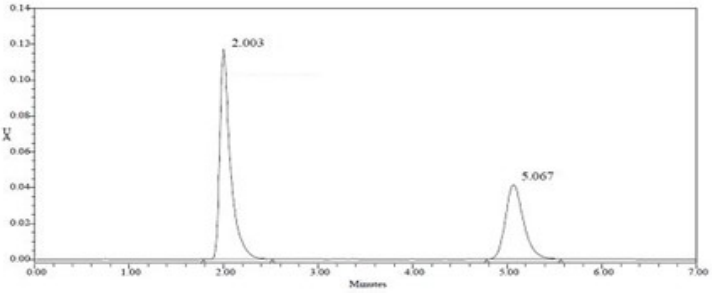

Figure 1 Chromatogram for system suitability

Table 1 Results of system suitability parameters for Rosuvastatin and Ezetimibe

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Rosuvastatin	2.003	920101	116666	1.5	1.6	2711.8

2	Ezetimibe	5.067	552058	41531	11.0	1.3	3428.2

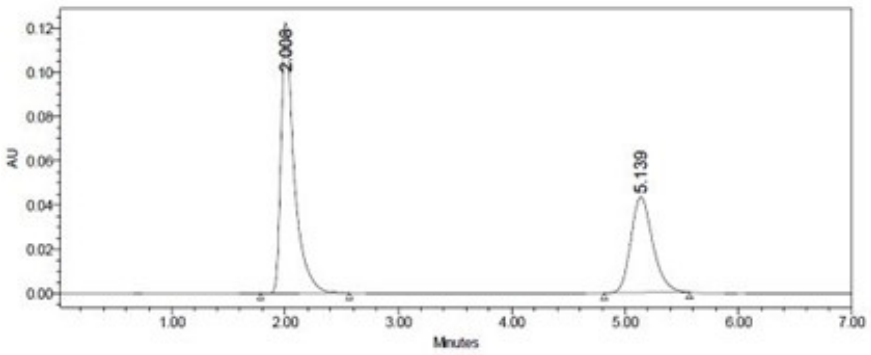

Figure 2 Chromatogram for sample injection

Table 2 Results of method precision for Rosuvastatin

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average	**983234**	**976311**	**100.84**
%RSD	**49.5**	**48.2**	**0.304**

Table 3 Results of method precision for Ezetimibe

S. No	Sample area	Standard area	Percentage purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average	**592325**	**582755**	**101.24**
%RSD	**29.5**	**28.7**	**0.46**

Table 4 Results of Intermediate precision for Rosuvastatin

S. No	Sample area	Standard area	Percentage purity
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average	**985321**	**984824**	**99.63**
%RSD	**48.2**	**48.5**	**0.54**

Table 5 Results of Intermediate precision for Ezetimibe

S. No	Sample area	Standard area	Percentage purity
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average	**596537**	**592542**	**99.27**
%RSD	**29.3**	**29.2**	**0.27**

Table 6 Results of Accuracy

Results of Accuracy		Rosu	Ezeti	Rosu	Ezeti	Rosu	Ezeti
50%	**1**	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					**99.7%**	**99.7%**
100%	**1**	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					**99.6%**	**99.8%**
150%	**1**	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					**99.7%**	**99.8%**

Table 7 Area of different concentration of Rosuvastatin and Ezetimibe

Concentration (µg/ml)	Peak area of Rosuvastatin	Peak area of Ezetimibe
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

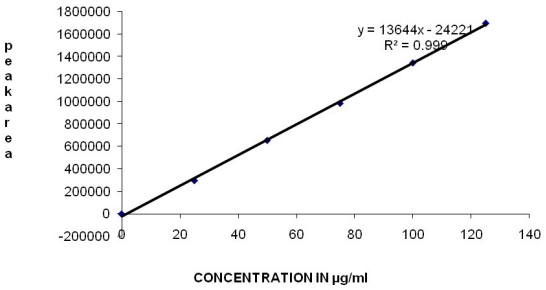

Figure 3: Calibration graph for Rosuvastatin

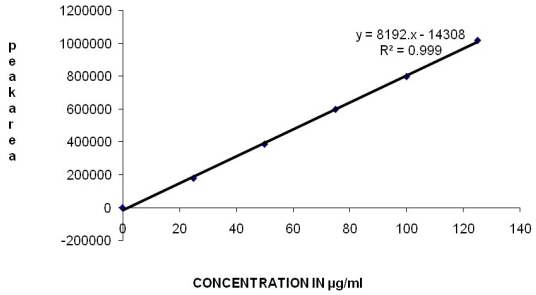

Figure 4: Calibration graph for Ezetimibe

Table 8 Analytical performance parameters of Rosuvastatin and Ezetimibe

Parameters	Rosuvastatin	Ezetimibe
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Table 9 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Rosuvastatin	56	176	3.14
Ezetimibe	56	154	2.75

Table 10 results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Rosuvastatin	56	563	10.05
Ezetimibe	56	558	9.96

Table 11 Results for effect of variation in flow

S. No	peak area for Less flow (0.7 ml/min)		peak area for More flow (0.9 ml/min)	
	Rosuvastatin	**Ezetimibe**	**Rosuvastatin**	**Ezetimibe**
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

Table 12 Results for effect of variation in mobile phase composition

S. No	peak area for Less organic(70%)		Peak area for More organic (90%)	
	Rosuvastatin	**Ezetimibe**	**Rosuvastatin**	**Ezetimibe**
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	**987286**	**583658**	**986641**	**592877**
%RSD	**0.45**	**0.90**	**0.51**	**0.57**

4. Conclusion

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Rosuvastatin and Ezetimibe in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Rosuvastatin and Ezetimibe was done by RP-HPLC. The Phosphate buffer was pH 2.5 and the mobile phase was optimized which consists of Acetonitrile:Phosphate buffer mixed in the ratio of 80:20 % v/v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 274 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Rosuvastatin and Ezetimibe were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Rosuvastatin and Ezetimibe LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Rosuvastatin and Ezetimibe in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

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