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Simultaneous Determination of Rosuvastatin Calcium and Amlodipine Besylate in Tablet Formulations by RP-HPLC Method

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay method has been developed for simultaneous estimation of Rosuvastatin calcium and Amlodipine besylate in tablet formulations. The separation was achieved by using YMC Pro C8 (150mm 4.6mm id, 5 µm particle size) column in mobile phase consisted of acetonitrile and 20mM potassium dihydrogen phosphate buffer pH adjusted to pH 3.5 with the help of dilute ortho-phosphoric acid in the ratio of (45:55 v/v). The flow rate was 1.0 mL.min-1 and the separated drugs were detected using UV detector at the wavelength of 242 nm. The retention time of Rosuvastatin calcium and Amlodipine besylate, was noted to be 4.42 and 2.91 respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible and consistent.

Keywords: Liquid Chromatography, Rosuvastatin calcium, Amlodipine besylate, combined dosage forms, Simultaneous estimation, Validation.

INTRODUCTION

Rosuvastatin calcium **Fig.1** is chemically, 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. Molecular formula of Rosuvastatin calcium is $(C_{22}H_{27}FN_3O_6S)_2$ Ca having molecular weight 1001.14 g/mole. Its melting point is 122º C [1-2].Rosuvastatin calcium is lipid lowering drug and use to prevent. It inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme that converts HMG-CoA to mevalonate a precursor of cholesterol and thereby checks the synthesis of cholesterol. It is used in the treatment of hyper-cholesterolemia and dyslipidemia [3-5].

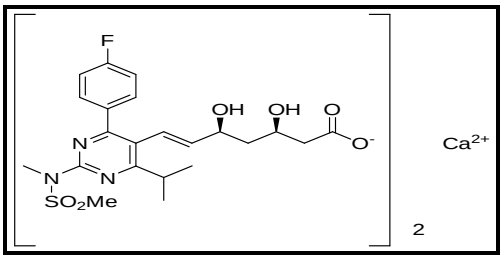

Fig.1 Chemical structure of Rosuvastatin calcium

Amlodipine besylate **Fig.2** is chemically described as 3-Ethyl-5-methyl (±)-2-[(2- aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3,5-pyridine-dicarboxylate, monobenzenesulphonate. Its empirical formula is $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$. Amlodipine besylate is a white crystalline powder with a molecular weight of 567.1. It is slightly soluble in water and sparingly soluble in ethanol [6-11]. Amlodipine besylate is a calcium channel blocker, which is used as an antihypertensive agent. It is official in EP and BP.

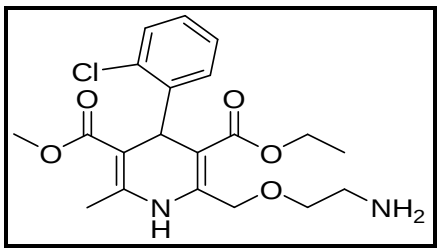

Fig.2 Chemical structure of Amlodipine besylate

Literature survey revealed a few HPLC [12-36] methods for the estimation of Rosuvastatin calcium and Amlodipine besylate. In the present study, an attempt has been made to develop a method for the simultaneous estimation of two drugs- Rosuvastatin calcium and Amlodipine besylate. It can also be applied for routine analysis of either one or of any combinations of in these drugs dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents: Acetonitrile and water (HPLC-grade) were from Spectrochem Pvt. Ltd. Mumbai, Hydrochloric acid and Orthophosphoric acid (AR Grade), Sodium hydroxide pellets were from Ranbaxy Fine Chemicals was obtained from Qualigens Ltd., Mumbai. Nylon syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation: The analysis of the drug was carried out on a waters LC system equipped with LC-10AT*vp* binary pump and an SPD-M10A*vp* photodiode array detector was used and a Reverse phase HPLC column YMC Pro C8 (150mm 4.6mm id, 5 µm particle size) was used. The output of signal was monitored and integrated using LC solution software.

Buffer preparation: 2.72g potassium dihydrogen phosphate was weighed and dissolves in 1000 ml Milli-Q water, pH adjusted to pH 3.5 with the help of dilute ortho-phosphoric acid. Filter the solution through 0.45µm membrane filter.

Mobile phase preparation: Prepare a filtered and degassed mixture of Buffer and acetonitrile in the ratio of 550:450 v/v. Filter the solution through 0.45µm membrane filter.

Diluent preparation: Mixed the solvents acetonitrile: water in the ratio 50:50 v/v.

Standard preparation: Stock solution of Rosuvastatin calcium (500µg/mL) and Amlodipine besylate (250µg/mL) was prepared by transferring accurately weighed 25 mg rosuvastatin and 12.5 mg amlodipine into a 50 mL volumetric flask and adding 20 mL water- acetonitrile (50:50 v/v). The mixture was sonicated for 2 min to dissolve the content and the solution was then diluted to volume with the same solvent mixture. Standard solution rosuvastatin calcium (100µg mL^{-1}) and Amlodipine besylate (50µg mL^{-1}) was prepared by diluting 10 mL standard stock solution to 50 mL in a volumetric flask with the same solvent mixture.

Sample preparation: To prepare stock solution (500 + 250µg mL^{-1}) for assay 20 tablets were weighed and mixed. An aliquot of powder equivalent to the weight of 5 tablets was accurately weighed and transferred to 100 mL volumetric flask. Water- acetonitrile (50:50 v/v), 60 mL was added to the flask and the mixture was sonicated for 10 min with normal hand shaking. The contents of the flask were then left to return to room temperature and diluted to volume with the same solvent mixture. This solution (20 mL) was filtered through a 0.45-µm nylon syringe filter. To prepare test solution (100 + 50 µg mL^{-1}) for assay 10 mL test stock solution was transferred to 50 mL volumetric flask and diluted to volume with water-acetonitrile (50:50, v/v).

Chromatographic conditions: Chromatographic analysis was performed on YMC Pro C8 (150mm 4.6mm id, 5 µm particle size) column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL min^{-1}. The sample injection volume was 20 µL. The photodiode array detector was set to a wavelength of 242nm for the detection and chromatographic runtime was 10 min.

RESULTS AND DISCUSSION

Method development: To develop a suitable and robust LC method for the determination of Rosuvastatin calcium and Amlodipine besylate, different mobile phases were employed to achieve the best separation and resolution. For mobile phase selection, preliminary trials using mobile phases of different composition containing water adjusted to acid pH by addition of orthophosphoric acid and methanol resulted in poor peak shape. When methanol and water were replaced by acetonitrile and phosphate buffer pH adjusted to pH 3.5 with the help of dilute ortho-phosphoric acid in the ratio of 45:55 v/v better peak shapes was obtained. The proportion of the mobile phase components was optimized to reduce retention times and enable good resolution between both molecules. The retention time of Rosuvastatin calcium is 4.42 min and Amlodipine besylate is about 2.91 (**Fig.3)** and the peak shape for these two was good. System suitability results of the method are presented in table-1**.**

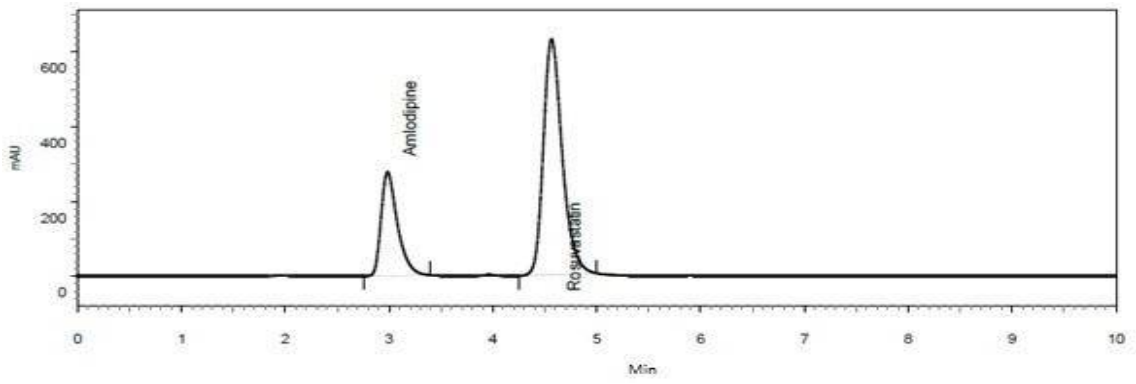

Fig.3 A typical HPLC Chromatogram showing the peak of Rosuvastatin calcium and Amlodipine besylate

Table 1. System suitability parameters for Rosuvastatin calcium and Amlodipine besylate by proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor	Resolution
Rosuvastatin calcium	4.42	9852	1.12	5.6
Amlodipine besylate	2.91	7885	1.16	-

Method validation: The developed RP-LC method extensively validated for assay of Rosuvastatin calcium and Amlodipine besylate using the following Parameters

Precision: In the study of the instrumental system precision where, a RSD of 0.76% was obtained for the standard area of Rosuvastatin calcium and 0.84% for Amlodipine besylate obtained corresponding to the first day, Similarly being 0.82% for Rosuvastatin calcium and 0.66% for Amlodipine besylate for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 0.76% for Rosuvastatin calcium. Similarly the method precision study for six sample preparations in marketed samples showed a RSD of 0.84% for Amlodipine besylate. The intermediate precision, a study carried out by the same analyst working on different day. The results (Table 2) calculated as inter-day RSD corresponded to 0.82 % for standard of Rosuvastatin calcium and 0.66% for standard of Amlodipine besylate. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Table 2 Method Precision studies for Rosuvastatin calcium and Amlodipine besylate by proposed method

Set	Rosuvastatin Calcium		Amlodipine Besylate	
	Intraday (n = 6)	Interday (n = 6)	Intraday (n = 6)	Interday (n = 6)
1	101.44	100.94	100.88	101.59
2	100.96	99.58	101.33	100.65
3	100.32	99.03	100.48	100.33
4	100.50	99.50	100.41	100.50
5	100.07	99.01	99.65	100.20
6	99.19	98.57	98.97	99.55
Mean	100.41	99.43	100.28	100.47
Std.Deviation	0.772	0.8825	0.8523	0.6673
%RSD	0.76	0.82	0.84	0.66

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Rosuvastatin calcium and Amlodipine besylate, analyzed as per the proposed method. The percentage recoveries found in the range of 98.96% and 101.94%, with an overall %RSD of 0.35 for Rosuvastatin calcium and The percentage recoveries with found in the range of 98.37% and 101.79%, with an overall %RSD of 0.45 for Amlodipine besylate. From the data obtained which given in table-3 and table-4 the method was found to be accurate.

Table 3. Recovery studies for Rosuvastatin calcium by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	101.87	0.09	0.35
100	99.56	0.54	
150	100.47	0.41	

Table 4. Recovery studies for Amlodipine besylate by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	101.17	0.94	0.45
100	98.50	0.11	
150	98.77	0.30	

Linearity of detector response: The standard curve was obtained in the concentration range of 40-160µg/ml for Rosuvastatin calcium and 20-80µg mL^{-1} for Amlodipine besylate. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in fig.4 for Rosuvastatin calcium and fig.5 for Amlodipine besylate to demonstrate the linearity of the proposed method. From the data obtained which given in Table 5 For Rosuvastatin calcium and Table.6 For Amlodipine besylate the method was found to be linear within the proposed range.

Table 5. Linearity studies for Rosuvastatin calcium by proposed method

Rosuvastatin calcium	
Conc.%	Area
40	2300158
60	3450237
80	4601136
100	5750395
120	6911474
140	8051373
160	9201272

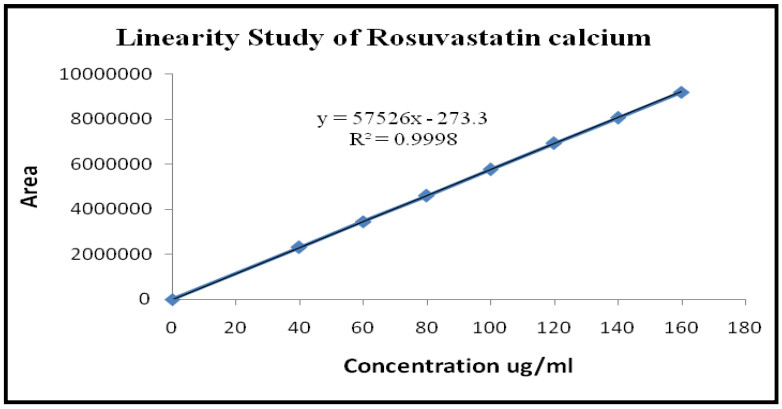

Fig 4. Calibration curve for Rosuvastatin calcium

Table 6. Linearity studies for Amlodipine besylate by proposed method

Amlodipine besylate	
Conc.%	Area
20	1014345
30	1521575
40	2028690
50	2535862
60	3043035
70	3550207
80	4057380

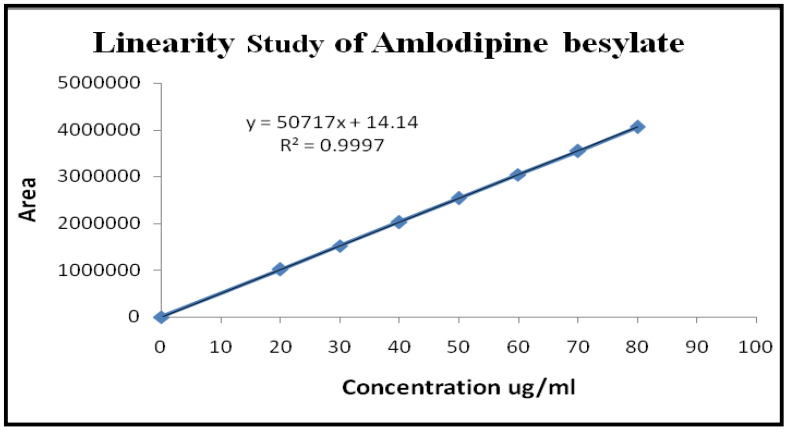

Fig 5. Calibration curve for Amlodipine besylate

APPLICATIONS

Proposed RP-HPLC method is useful for the determination in the pharmaceutical dose form of drugs. It is easy, simple, accurate and reproducible method for determination of Rosuvastatin calcium and Amlodipine besylate with comparing the existing RP-HPCL method.

CONCLUSIONS

RP-HPLC method for simultaneous estimation of Rosuvastatin calcium and Amlodipine besylate was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 40-160µg/ml for Rosuvastatin calcium and 20-80µg/mL for Amlodipine besylate. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of

formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision.

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