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Research Article

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Development and validation of a RP-HPLC-PDA method for simultaneous determination of Rosuvastatin calcium and Amlodipine besylate in pharmaceutical dosage form

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ABSTRACT

RP-HPLC-PDA method has been developed and validated for quantitative determination of ROSUVASTATIN and AMLODIPINE from tablet formulations. All the parameters for the two titled drugs met the criteria of ICH guidelines for method validation. As the mobile phase is MS compatible then method can be used to determine analytes individually or in combination in biological fluids to study the pharmacokinetics and used for LC-MS system. The method is very simple, rapid and economic in nature as all peaks are well separated, which makes it especially suitable for routine quality control analysis work. Symmetrical peaks were obtained through experimental trials. Two columns were used for performance investigations, including Kromasil C_{18} (5 micron 4.6 × 250mm) and Qualisil C_8 (5 micron 4.6 × 250mm), the first column was the most suitable one since it produced symmetrical peaks with high resolution. The UV detector response of ROSUVASTATIN and AMLODIPINE was studied and the best wavelength was found to be 251 nm showing highest sensitivity. Several modifications in the mobile phase composition were made in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, pH, flow rate, temperature and stability of ROSUVASTATIN and AMLODIPINE were also studied in methanol and mobile phase.

Keywords: - RP-HPLC, Rosuvastatin, Amlodipine

INTRODUCTION

Rosuvastatin Calcium (ROSU), 7-{4-(4-Fluorophenyl)-6-isopropyl-2-[methyl(methyl sulfonyl)amino]pyrimidin-5-yl}-3,5-dihydroxyhept-6-enoic acid is a member of the drug class of statins, is a competitive inhibitor of HMG-COA reductase enzyme. It is mainly used for treatment of hypercholesterolemia and prevention of cardiovascular disease. Rosuvastatin calcium is official in Indian pharmacopoeia and Martindale, the extra pharmacopoeia [1-3]. Few UV spectrophotometric, HPLC and HPTLC methods have been reported individually or in combination with other drugs for estimation of Rosuvastatin calcium. Amlodipine besylate is chemically designated as 3-ethyl 5-methyl2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-l, 4-dihydro-6-methyl-3, 5pyridine dicarboxylate benzene sulphonate. It is the besylate salt of Amlodipine, a long-acting calcium channel blocker used in treatment of hypertension and coronary artery diseases. Amlodipine besylate is official in Indian pharmacopoeia, Martindale, The extra pharmacopoeia, European pharmacopoeia and United states pharmacopoeia. Various UV, HPLC, HPTLC and stability indicating methods for Amlodipine besylate have been reported individually or in combination with other drugs [4-10]. To our knowledge there is no HPLC method reported for the combination, availability of an HPLC method with high sensitivity and selectivity will be very useful for the estimation of ROSUVASTATIN and AMLODIPINE in combined pharmaceutical dosage forms. Therefore the aim of the study was to develop and validate sensitive, precise, accurate and specific HPLC method for the determination of ROSUVASTATIN and

AMLODIPINE simultaneously in formulation as per ICH guidelines . The present work describes a simple reverse phase LC method for the determination of ROSUVASTATIN and AMLODIPINE in tablets.

EXPERIMENTAL SECTION

***Materials and Reagents*:**
Two tablet formulations from Jalgaon Chemicals Pharma Ltd, Jalgaon (Formulation I, Batch No. JT 985) and (Formulation II, Batch No. JT 987) containing Rosuvastatin Calcium (ROSU) 10 mg and Amlodipine Besylate (AMLO) 5 mg per tablet were used for analysis. Pure drug sample of ROSU, % purity 98.5 and AMLO, % purity 99.91 was kindly supplied as a gift sample by Glenmark Generic Ltd., Mumbai and Emcure Pharmaceutical Ltd., Pune, respectively. These samples were used without further purification. HPLC grade methanol, tetrahydrofuran and acetonitrile were procured from Merck Chemicals (Mumbai, India), Qualigens Fine Chemicals (Mumbai, India) and Thomas Baker (Mumbai, India) respectively. AR grade ortho phosphoric acid was procured from Research Lab Fine Chem. (Mumbai, India). Double distilled water and placebo tablets were made at Lab scale only.

***Instrumentation and Chromatographic Conditions*:**
The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater and PDA detector (Waters 2998). Data collection and analysis were performed using Empower - version 2 software. Separation was achieved on Kromasil C_{18} column (250 mm × 4.6 mm, 5.0 µ) column maintained at 50^0C using column oven. Isocratic elution with acetonitrile: THF: water pH 3 (68:12:20 % v/v) mobile phase at the flow rate of 0.5 ml/min was carried out. The detection was monitored at 251 nm and injection volume was 10 µl. The peak purity was checked with the PDA.

***Preparation of Standard Solutions and Calibration Curve*:**
Standard stock solution of ROSUVASTATIN AND AMLODIPINE (1000 µg/ml) were prepared separately in methanol. From these solutions 100 µg/ml concentration solution prepared in 10ml volumetric flask. For analysing the linearity range of each component serial dilutions of ROSUVASTATIN AND AMLODIPINE were made from 1.0 to 160 µg/ml and 0.5 to 80 µg/ml, respectively in mobile phase and injected onto column. Calibration curves were plotted as concentration of drugs versus peak area response. The system suitability test was performed from six replicate injections of mixed standard solution. The baseline separation of mixture and specificity of ROSUVASTATIN and AMLODIPINE was given in [**Figure 1 and Table 1**] respectively.

Analysis of Tablet Formulations:
Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 10 mg of ROSU (5 mg of AMLO) was weighed and dissolved in the 80 ml of methanol with the aid of ultrasonication for 15 min and solution was filtered through Whatmann paper No. 41 into a 100 ml volumetric flask. Filter paper was washed with the methanol, adding washings to the volumetric flask and volume was made up to mark. From the filtrate, appropriate dilution was done in mobile phase to get a solution of 100 µg/ml of ROSU. From this solution appropriate dilutions were made and injected into the system to get the chromatogram [**Figure 2**]

METHOD DEVELOPMENT:
The HPLC method was validated in terms of linearity, precision, accuracy, robustness, LOD and LOQ according to ICH guidelines. [15-17]

Linearity, Range and Method sensitivity
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analytes in the sample. For the construction of calibration curves, eight calibration standard solutions were prepared over the concentration range. The values of limit of detection(DL) and limit of quantitation (QL) were calculated by using standard deviation of the response (σ) and slope of calibration curve of analyte (S) and using formula DL = 3.3 σ/S and QL = 10 σ/S

Precision
The precision of repeatability was studied by replicate (n=3) analysis of tablet solutions. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation.

Accuracy
The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Recovery studies were carried out by applying the method to drug content present in tablet dosage form to which known

amount of mix standard of ROSUVASTATIN AND AMLODIPINE was added at 50 %, 100 % and 150 % levels. The base level Selected was 50µg/ml and 25µg/ml of ROSUVASTATIN AND AMLODIPINE, respectively. The technique involves addition of standard drug solution to preanalysed sample solution. The resulting sample solutions were injected and chromatograms were recorded and the concentrations of both the standard drugs from tablet sample were determined using the respective calibration graphs. At each of the levels, three determinations were performed and results were obtained.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The flow rate was varied by (±) 0.05 ml/min, the percentage of acetonitrile was varied by (±) 5 %, column temperature was varied by (±) 2° C, pH of mobile phase was varied by (±) 0.2, the column was changed from different lots and wavelength of measurement was changed by (±) 1nm. One factor at the time was changed to estimate the effect. The solutions containing 100 µg/ml of ROSU and 50 µg/ml of AMLO were injected in the column. A number of replicate analyses (n = 3) were conducted at 3 levels of the factor (-, 0, +).

Solution Stability

The stability of the drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 hrs and then analyzing. The long-term stability was determined by storing at 4 ^{0}C for 30 days. Auto-sampler stability was determined by storing the samples for 24 hrs in the auto-sampler. For method development and optimization, retention factor (*k*) was calculated using the equation: $k = (t_R - t_M)/ t_M$. Where, t_R = retention time, t_M = is the elution time of the solvent front.

RESULTS AND DISCUSSION

Method Optimization:

Symmetrical peaks were obtained through experimental trials. Two columns were used for performance investigations, including Kromasil C_{18} (5 micron 4.6 × 250mm) and Qualisil C_8 (5 micron 4.6 × 250mm), the first column was the most suitable one since it produced symmetrical peaks with high resolution. The UV detector response of ROSUVASTATIN and AMLODIPINE was studied and the best wavelength was found to be 251 nm showing highest sensitivity.

Several modifications in the mobile phase composition were made in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, pH, flow rate, temperature and stability of ROSUVASTATIN and AMLODIPINE were also studied in methanol and mobile phase.

Initially methanol and water in different ratios were utilized, but both drugs showed peak broadening and the poor resolution. So methanol was replaced by acetonitrile ,both drugs showed good peaks but with the problem of tailing so THF was added to reduce the tailing and water was used with different pH. The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 3.0-6.0. At PH 3.0 was the most appropriate one giving well-resolved peaks and highest no. of theoretical plates. The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 60-40 % acetonitrile. Table 1 shows that 68 % acetonitrile was the optimum one giving well resolved peaks and higher no. of theoretical plates. The effect of flow rate on the formation and separation of peaks was studied by varying the flow rate from 0.5-1.0 ml/min; a flow rate of 0.6 ml/min was optional for good separation and resolution of peaks in a reasonable time shown in [**Table 1 and Figure 1]**

METHOD VALIDATION:

The method was validated, in accordance with ICH guidelines, for linearity, range, accuracy, precision, LOD and LOQ, specificity, ruggedness and robustness.

Linearity and range:

Linearity was determined for ROSU in the range of 1-160 µg/ml and for AMLO 0.5-80.0 µg/ml. The correlation coefficient ('r^2') values were > 0.999 (n = 6). Typically, the regression equations for the calibration curve was found to be y = 41557.03x – 13194.6 for ROSU, y = 24216.52X+3160.742 for AMLO. Excellent correlation exists between response factor and concentration of drugs within the concentration range..

Formulation Analysis
The assay for the marketed tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 99.88 % for ROSU and 99.41 % for AMLO of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results are given in **[Table 2 and 3]**

Precision:
The precision of the method was done by replicate (n=3) analysis of tablet preparations. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in [**Table 4 and 5]**

Accuracy:
Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The resulting sample solutions were injected and chromatograms were recorded. The mean percentage recoveries obtained for ROSUVASTATIN AND AMLODIPINE were 99.55 % and 99.67 %, respectively, reported in [**Table 6].**

Robustness:
Robustness of the method was determined by making slight changes in the chromatographic conditions. The % Assay and % RSD was found to be in range 100 ± 1.5 % and < 2, respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):
The LOD and LOQ values were found to be 0.11 and 0.34 µg/ml and 0.06 and 0.19 µg/ml for ROSUVASTATIN and AMLODIPINE, respectively.

Specificity:
In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes. This indicated that the peak of analytes was pure and excipients in the formulation did not interfere with the analytes.

Solution Stability Studies:
Stability as described in method development under experimental section was studied. Result of short-term, long-term and the auto sampler stability of the ROSUVASTATIN and AMLODIPINE solutions were calculated from nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (98–102%).

Table 1: System suitability parameters with peak purity data

Parameter		AMLO	ROSU
Retention time (t_R)		3.7	5.4
USP Resolution[a] (R_S)		-	3.82
Tailing factor [a] (T)		1.1	1.14
No. of therotical plates[a] (N)		4128	5010
Capacity Factor (k' prime)		2.92	4.48
Peak Purity Data	Peak Angle	0.235	0.256
	Peak Threshold	0.567	0.614

Table 2: Analysis of Tablet Formulation I

Sr. No.	Label Claim (mg/tab)		% of Label claim determined,% RSD	
	ROSU	AMLO	ROSU	AMLO
1	10	5	101.03, 1.23	99.05, 0.83
2	10	5	100.7, 1.07	98.87, 0.49
3	10	5	98.92, 0.23	100.07, 1.29
4	10	5	99.28, 0.57	98.93, 0.62
5	10	5	99.37, 0.74	101.17, 1.43
6	10	5	100.24, 0.92	99.41, 0.97

Table 3: Analysis of Tablet Formulation II

Sr. No.	Label Claim (mg/tab)		% of Label claim determined, % RSD	
	ROSU	AMLO	ROSU	AMLO
1	10	5	100.72, 1.24	100.21,0.64
2	10	5	98.95, 0.42	98.84, 0.32
3	10	5	99.28,0.71	100.37,0.92
4	10	5	99.63, 0.82	98.62, 0.52
5	10	5	100.28, 0.97	98.51,0. 47
6	10	5	100.81, 1.45	99.72,0.79

Table 4 : Intraday and Inter day precision of ROSU (n=3)

ROSU	Measured concentration (µg/ml), % R.S.D	
Conc. (µg/mL)	Intra day	Inter day
10	10.01,1.47	10.15,1.32
100	99.79, 0.82	100.07, 0.86
200	200.01,0.53	200.12, 0.38

Table 5: Intraday and Inter day precision of AMLO (n=3)

AMLO	Measured concentration (µg/ml), % R.S.D	
Conc. (µg/mL)	Intra day	Inter day
5	5.47, 0.89	5.04, 1.65
50	50.12, 0.45	50.27, 0.82
100	100.03,0.32	100.67, 0.47

Table 6: Accuracy (recovery) of ROSUVASTATIN AND AMLODIPINE

Compound	Recovery Level (%)	Qty. spiked (µg/mL)	Qty. recovered (µg/mL)	Recovery (%)	R.S.D (%)
ROSU	50	25	24.64	98.56	0.52
	100	50	49.27	101.48	0.84
	150	75	74.53	100.63	0.32
AMLO	50	12.5	12.29	101.70	0.76
	100	25	25.32	98.73	0.49
	150	37.5	37.81	99.18	0.81

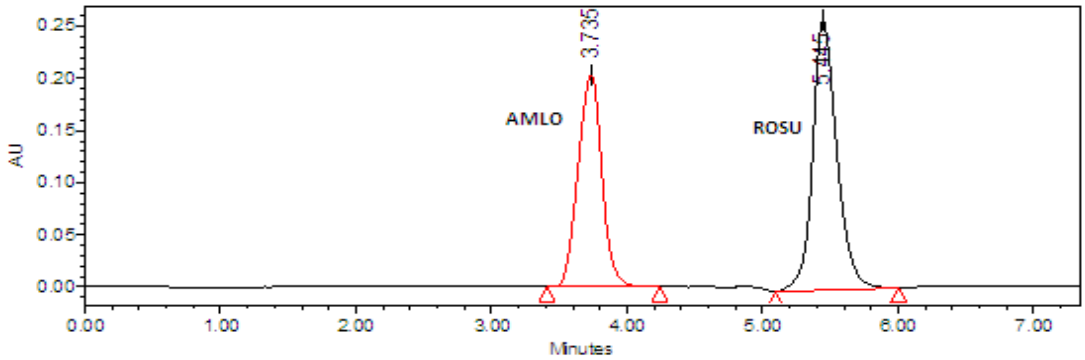

Figure 1: Chromatogram for working standard mixture of ROSU 100 µg/mL & AMLO 50 µg/mL

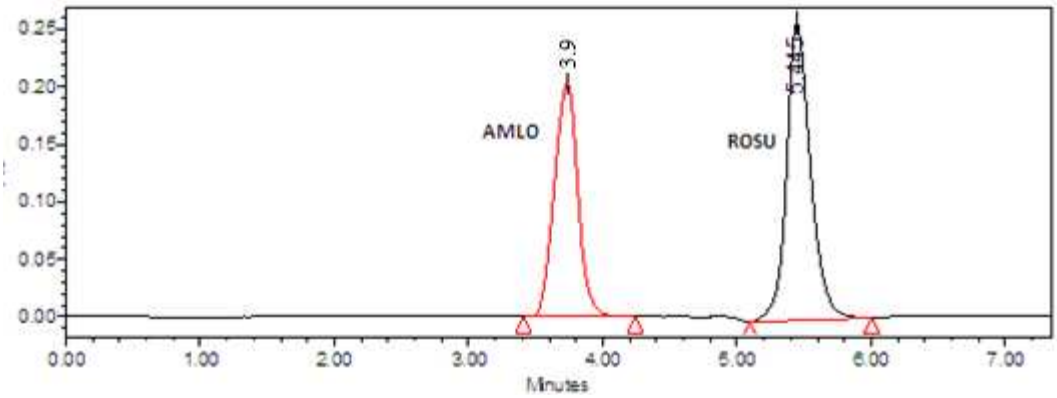

Figure2: Chromatogram of the tablet formulation (100µg/mL of ROSU & 50µg/mL of AMLO)

CONCLUSION

Linear, precise, and accurate RP-HPLC-PDA method has been developed and validated for quantitative determination of ROSUVASTATIN and AMLODIPINE from tablet formulations. All the parameters for the two titled drugs met the criteria of ICH guidelines for method validation. As the mobile phase is MS compatible method can be used to determine analytes individually or in combination in biological fluids to study the pharmacokinetics and used for LC MS system. The method is very simple, rapid and economic in nature as all peaks are well separated, which makes it especially suitable for routine quality control analysis work.

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