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RP-HPLC method development for the simultaneous estimation of atorvastatin and amlodipine besylate in bulk and pharmaceutical dosage forms

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ABSTRACT

The present study was aimed to develop a high-performance liquid chromatographic (HPLC) method for atorvastatin and amlodipine besylate with high sensitivity, accuracy and precision in combined dosage form. Separation was performed on a C18 column [Agilent ODS UG 5 column, 250mm x 4.5mm], with Phosphate buffer (pH 3.4): Acetonitrile (50:50) isocratic elution with a flow rate of 1ml/min. Good sensitivity was observed with UV detection at 259nm. After method development, the interference of other active compounds and excipients, repeatability and linearity were investigated. Retention times of atorvastatin and amlodipine besylate were found to be 6.8 and 8.6 min respectively. The method was validated over the range from 100-500µg/mL for atorvastatin and 50-250 µg/mL for amlodipine besylate with correlation coefficients of 0.9997 and 0.9996 respectively. This method was shown to be accurate, robust, selective, linear, and repeatable and can be successfully employed in routine quality control for the simultaneous analysis of atorvastatin and amlodipine besylate in tablets.

Key words: Atorvastatin(ATR), amlodipine besylate (AMB), RP-HPLC, Validation.

INTRODUCTION

Atorvastatin Calcium is chemically (Fig 1) (3R, 5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid. It is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis [1]. It is used to reduce high (harmful) levels of low-density lipoprotein cholesterol (LDL-C) in the blood. Amlodipine besylate (AMB) is chemically (Fig 1) 3-Ethyl 5-methyl 2-(2-aminoethoxymethyl)4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate monobenzenesulphonate. It is a Amlodipine besylate is a dihydropyridine calcium-channel blocker [2] AMB is official in British Pharmacopoeia [3]. The combination dosage forms of atorvastatin calcium and amlodipine besylate are available in the market for the treatment of hypertension, chronic stable angina, vasospastic angina, elevated serum triglyceride levels, primary dysbetalipoproteinemia. Literature revealed that only few methods have been reported for the individual estimation of ATR and AMB and in combination [4-8]. The present study is to develop a precise, accurate, simple, economical and specific RP-HPLC method to determine ATR and AMB in pharmaceutical dosage forms.

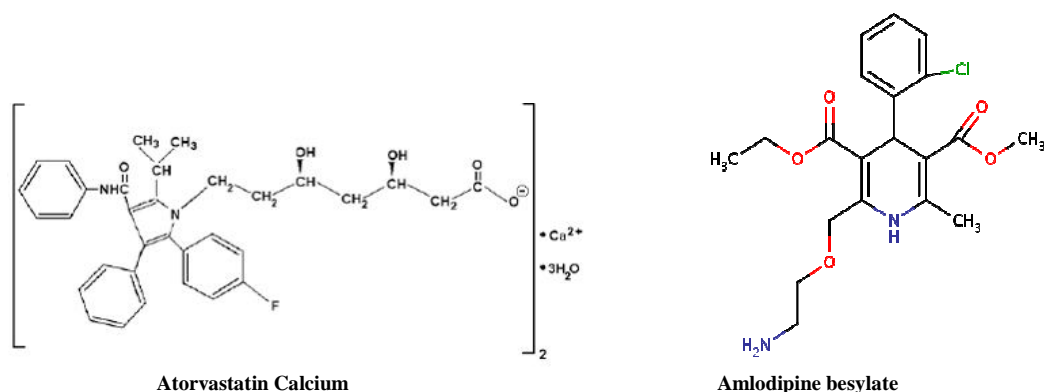


Fig. 1: Chemical structures of ATR and AMB

MATERIALS AND METHODS

Equipment

Agilent 1120 compact LC chromatographic system, equipped with variable wavelength programmable UV detector and Rheodyne injector with 20 μ L fixed loop was used for the chromatographic separation. The chromatogram was recorded and peaks were quantified by means of Ezchrome software. Chromatographic separation was carried out on a C18 column [Agilent ODS UG 5 column, 250mm x 4.5mm]. Elico double beam SL 218 UV-Visible spectrophotometer for spectroscopic determinations and Axis AGN 204-PO electronic balance was used for weighing purpose.

Chemicals and Reagents

Atorvastatin (ATR) and amlodipine besylate (AMB) were procured as gift samples from Aurobindo Pharma Ltd., Hyderabad and Matrix Laboratories Ltd., Hyderabad respectively. The tablet dosage form containing Atorvastatin and Amlodipine besylate (AMTOR 10) were procured commercially from the local market. HPLC grade Acetonitrile and water procured from Merck, India.

Chromatographic Conditions

Mobile phase consisting of Phosphate buffer pH 3.4 : Acetonitrile (50:50) was used in isocratic mode. The mobile phase was initially filtered through 0.45 μ m millipore membrane filter and sonicated for 15 min before use. The flow rate was maintained at 0.8 mL/min and the injection volume was 20 μ L. UV detection was performed at 259 nm and the separation was achieved at ambient temperature.

Experimental

Mobile phase composition:

Mobile phase consisted of Phosphate buffer pH 3.4 : Acetonitrile (50:50) ratio was used for the present experimentation.

Preparation of standard stock solution

The separate stock solutions of Atorvastatin and Amlodipine besylate were prepared by accurately weighing 25 mg each into a separate 25 ml volumetric flasks A and B and made up to the volume with mobile phase to get 1000 μ g/ml respectively. From the above standard stock solutions 1mL from volumetric flask A and 1ml from volumetric flask B was transferred to a 10 ml volumetric flask and made up to the volume with same mobile phase to get 100 μ g/ml and 100 μ g/ml of Atorvastatin and Amlodipine besylate (Working stock solution). The stock solution was filtered through 0.45mm Millipore membrane filter, sonicated and degassed.

Preparation of sample solution

The tablets (Amtor10) were initially powdered and an amount equivalent to 25 mg of Atorvastatin and 25 mg of Amlodipine besylate was accurately weighed into a 25ml volumetric flask, mixed with 25 ml of mobile phase. The solution was made up to the volume with mobile phase and sonicated for 5 minutes. The solution was then filtered

through 0.45µm millipore membrane filter. Final stock containing 500µg/ml and 250µg/ml of Atorvastatin and Amlodipine besylate respectively was prepared by subsequent dilution with the same mobile phase.

METHOD VALIDATION

The method was validated according to ICH Q2 B guidelines for validation of analytical procedures in order to determine system suitability, linearity, sensitivity, precision, accuracy and robustness for the analytes [9].

System suitability

System suitability was carried out by injecting 100% concentration (sample having 500µg/ml of Atorvastatin and 250µg/ml of Amlodipine besylate) into the HPLC system. This was repeated for six times under similar condition. The tailing factor (T) and no. of theoretical plates (N) obtained were given in Table 1.

Linearity

Linearity of the method was determined by means of calibration curve using different concentration of the drugs. Linearity was evaluated by visual inspection of a calibration curve. The linearity of the method was determined in concentration range of 10-100µg/ml for Atorvastatin and 5-100µg/ml for, Amlodipine besylate. Each solution was injected in triplicate. The slope, intercept was reported as required by ICH. The calibration curves for ATR and AMB were shown in Figure 3a and 3b and their corresponding linearity parameters were given in Table 2.

Accuracy, as recovery

To confirm the accuracy of the proposed method, recovery experiments were performed by standard addition technique. In this method a known quantity of pure drug was added at three different levels i.e. 80 %, 100% and 120% to pre-analyzed sample solutions and calculated the recovery of ATR and AMB for each concentration. The results were given in Table 4.

Precision

Precision was studied by measuring intra-day (repeatability which was carried out by analyzing the drug solutions within same day) and inter-day (by injecting of samples over two consecutive days) variation of the method. Study was carried out by injecting six replicates of 100% concentration (500µg/mL of ATR and 250/mL of AMB) and the % RSD of the peak areas were given in Table 3.

Selectivity/Specificity

Selectivity is the ability of the method to produce a response for the analyte in the presence of other interferences, in order to prove that the method chosen was specific and selective. The parameters like retention time (R_t), resolution (R_s), tailing factor were calculated and given in Table 1.

LOD and LOQ

The LOD and LOQ values were determined by the formulae $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$ (Where, σ is the standard deviation of the responses and S is mean of the slopes of the calibration curves) and were given in Table 2.

Robustness

The robustness of the method was investigated under a variety of conditions including changes in the flow rate and detection wavelength and the % RSD was given in Table 5.

RESULTS AND DISCUSSION

The scope of the present work was to optimize the chromatographic conditions for the estimation of ATR and AMB in selected multicomponent dosage forms by RP-HPLC method. A binary mixture of Phosphate buffer pH 3.4 : Acetonitrile (50:50) as mobile phase was proved to be most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing. The developed method was also validated. The retention times obtained FOR atorvastatin and amlodipine besylate were found to be 6.8 and 8.6 min respectively. An optimized chromatogram showing the separation of ATR and AMB at different R_t 's were shown in Figure 2

Table 1: System suitability parameters

Parameters	Atorvastatin	Amlodipine
Retention time (min)	6.4	8.7
Theoretical plates (N)	7251	11230
Tailing factor (T)	1.3	1.1
Resolution (R _s)	1.68	

Table 2: Results for Linearity (n=3)

Parameters	Atorvastatin	Amlodipine
Slope	10239	64428
Intercept	71133	11800
r ²	0.996	0.998
Regression equation	y = 10239x + 71133	y = 64428x + 11800
Linearity range	10-100 µg/ml	5-100 µg/ml
LOD	0.67 µg/ml	0.54 µg/ml
LOQ	1.99 µg/ml	1.64 µg/ml

*n= No. of determinants

Table 3: Results for Precision (n=6)

Drug	Intraday Precision (% RSD)	Interday Precision (% RSD)
Amlodipine	0.95	0.58
Atorvastatin	0.58	0.89

*n= No. of determinants

Table 4: Results for Accuracy (n=3)

Spiked level	Mean % recovery		Mean %RSD	
	Atorvastatin	Amlodipine	Atorvastatin	Amlodipine
80	99.76	98.80	0.61	0.64
100	99.65	99.65	0.63	0.28
120	101.18	99.58	0.56	0.82

*n= No. of determinants

Table 5: Results for Robustness

Parameters (n=3)	Standard Deviation		% RSD	
	Atorvastatin	Amlodipine	Atorvastatin	Amlodipine
At λ max 257 nm	0.00650	0.002081	0.10	0.33
At λ max 261 nm	0.00832	0.002081	0.23	0.43
With flow rate 0.6 ml	0.00305	0.00305	0.59	0.43
With flow rate 1.0 ml	0.00305	0.00360	0.54	0.54

*n= No. of determinants

Table 6: Results for Assay (n=3) of marketed formulation

Drug	Label claim (mg/tab)	Amount recovered	% Amount found in drug
Atorvastatin	5 mg	4.86 mg	99.31 % w/w
Amlodipine	10 mg	10.48 mg	101.1% w/w

*n= No. of determinants

System suitability was carried out by injecting 100% concentration of ATR and AMB six times into the HPLC system. The tailing factor was less than 2 and theoretical plate number was more than 2000 for both the drugs were within the limits. The parameters were given in Table 1.

The linearity and range was found be in the range of 10-100 µg/mL for ATR and 5-100 µg/mL for AMB. The correlation coefficient of ATR and AMB were found to be 0.999 and 0.999 respectively and thus indicated the good linearity in the specified concentration range. The results were given in Table 2.

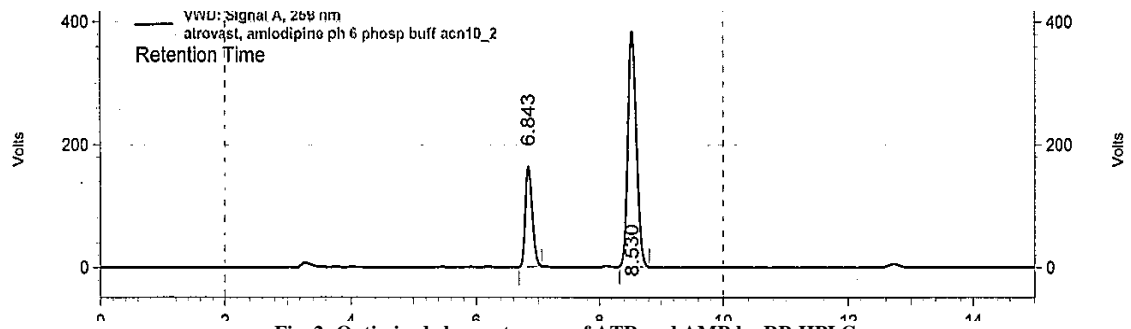


Fig. 2: Optimized chromatogram of ATR and AMB by RP-HPLC

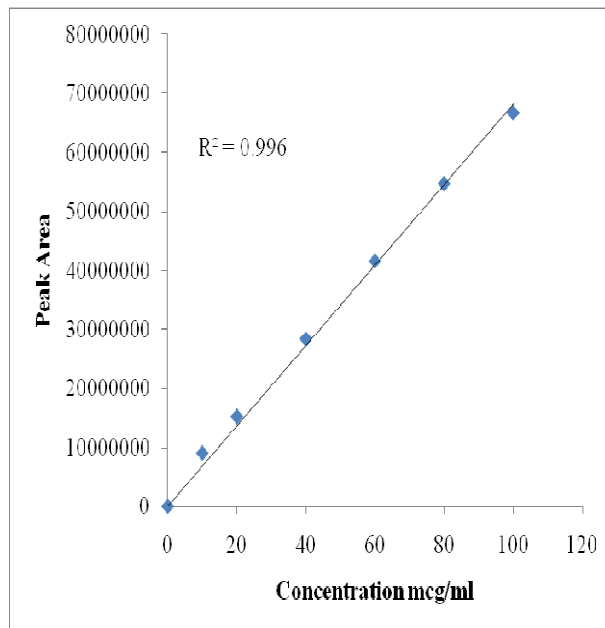


Fig. 3 a: Calibration Curve of Atorvastatin

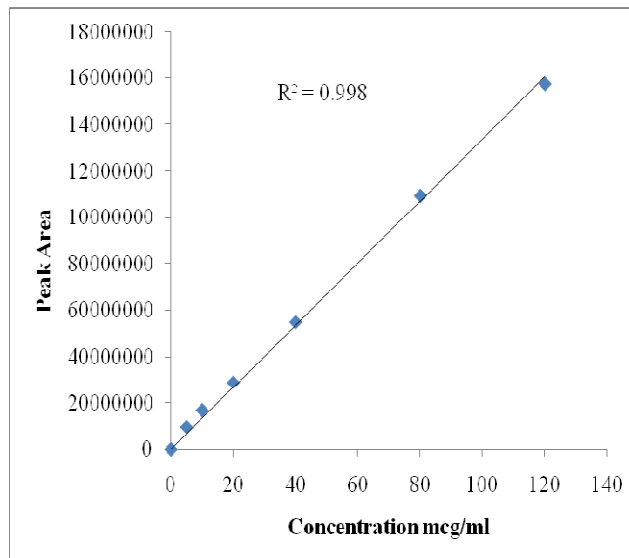


Fig.3 b: Calibration Curve of Amlodipine besylate

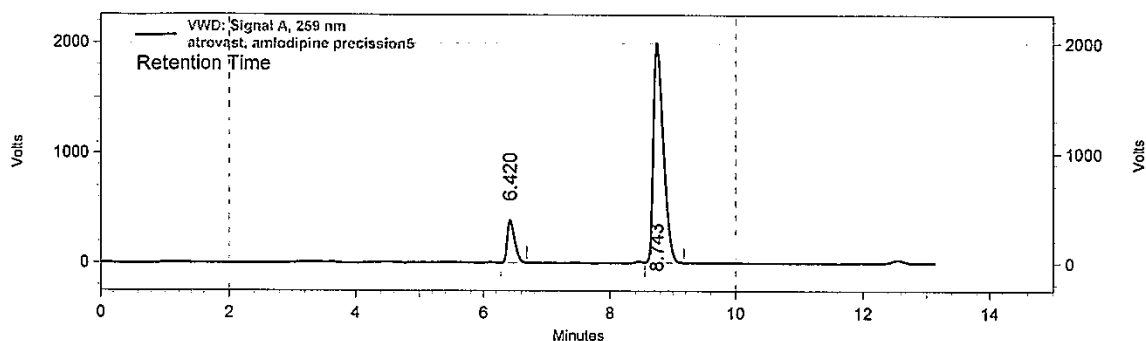


Fig. 4: A typical chromatogram Test Formulation (AMTOR 10)

Precision was reported as % RSD and given in Table 3. The minimum variation in the %RSD values obtained indicated that the present method is precise.

Accuracy of the proposed method was assessed by standard addition method at 80%, 100% and 120% levels of recovery to the preanalysed sample in triplicate. Recovery values obtained were given in Table 4 indicated that the proposed method was highly accurate. The recovery of the added standard to the drug product sample was calculated and was found to be 99.16, 99.65 and 100.18 % for ATR and 99.80, 99.60 and 101.2% for AMB. The % RSD was less than 2 for both the drugs which indicated a good accuracy of the method to that of the label claim.

The method specificity was assessed by studying the chromatograms obtained from the sample solution. The method was found to be specific as none of the excipients interfered with the analytes of interest which was shown in Figure 4. Hence, the method was found to be suitable for analyzing the commercial formulations.

LOD and LOQ were calculated from the average slope and standard deviation of y-intercepts of the calibration curve. LOD was found to be 0.41 $\mu\text{g/mL}$ and 0.38 $\mu\text{g/mL}$ respectively for ATR and AMB, LOQ was found to be 1.23 $\mu\text{g/mL}$ and 1.03 $\mu\text{g/mL}$ respectively for ATR and AMB indicated high sensitivity of the method. The results were given in Table 2.

Robustness was carried out by change in the flow rate ($\pm 0.1\text{mL/min}$) and variation in wavelength ($\pm 2\text{ nm}$). Solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D calculated was found to be less than 2 for each condition. The results were given in the Table 5.

Assay of marketed formulation:

20 μl of sample solution was injected into chromatographic system and the peak responses were measured. The solution was injected three times into the column. The amount present in each tablet was calculated by comparing the areas of test with that of the standard and found to be 99.80 % w/w and 101.0 %w/w for ATR and AMB respectively. A typical chromatogram of test solution containing 50 $\mu\text{g/ml}$ of ATR and 100 $\mu\text{g/ml}$ of AMB was shown in Figure 4. The results were given in Table 6.

CONCLUSION

The developed and validated RP-HPLC method was found to be economical due to the use of higher percentage of water as a solvent in mobile phase. The low solvent consumption (1 mL/min), along with short analytical run time of less than 10.0 minutes lead to an environmental friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. This method has been found to be better than previously reported methods, due to its wider range of linearity, use of readily available mobile phase, lack of extraction procedures. Hence above method can be used in quality control for routine analysis of finished products of ATR and AMB simultaneously without any interferences.

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