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RP-HPLC Method for Simultaneous Determination of Amlodipine Besylate and Atorvastatin Calcium: Application to Commercially available drug products

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ABSTRACT
A Reverse phase method has been developed for the quantitative estimation of Amlodipine Besylate and Atorvastatin Calcium in Tablets. The Quantification was carried out using RP stainless steel column ODS C18 250 x 4.6 x 5 µ L1 packing in Isocratic mode with mobile phase containing 0.03 M Potassium buffer: Acetonitrile in the ratio of 30:70, pH 2.5 adjusted with ortho-phosphoric acid. Solution degassed before use, Flow rate was maintained at 1.0 ml/minute and the detection wavelength set at 237 nm. The linearity was found to be in the range of 16-22 µg/ml for Atorvastatin Calcium and 4 to 6 µg/ml for Amlodipine Besylate .The proposed method found to be simple, precise, accurate, and reproducible for the estimation of Amlodipine Besylate and Atorvastatin Calcium.
Key words: *- Atorvastatin Calcium, Amlodipine Besylate, Method Development, High Performance Liquid Chromatography.*

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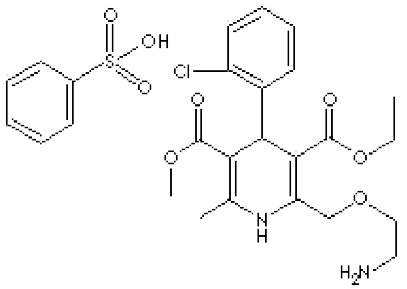
INTRODUCTION

Amlodipine Besylate

Amlodipine Besylate is one of the Calcium channel blockers work's primarily to relax the arterial muscles dilates and drops the blood pressure .Hypertension (blood pressure dropping too low) generally does not occur with Amlodipine Besylate unless it is combined with another drug that drops blood pressure [1,2].

Figure 1: Chemical structure of Amlodipine Besylate.



Atorvastatin

Atorvastatin (Fig 2.) is [R-(R*, R*)]-2-(4- flurophenyl)-B, B—dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoicacid.It is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) and a competitive inhibitor of HMG-CoA reductase. Catalyzes the reduction of 3-hydroxy-3-methylgultaryl-coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. [3, 4]

Figure 2 : Chemical structure of Atorvastatin Calcium.

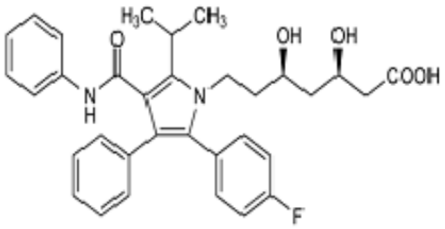


Fig. 2. Atorvastatin

EXPERIMENTAL

Material and reagents

Amlodipine Besylate procured from Cipla Ltd., Kurkumbh, and Atorvastatin Calcium from SL Drugs and Pharmaceuticals Limited from Hyderabad, Acetonitrile (HPLC grade) purchased from Qualigens Fine Chemicals (Mumbai, India). Sodium Hydroxide, Hydrochloric acid and Hydrogen Peroxide obtained from Merck Laboratories Ltd., (Mumbai, India). Double-distilled water used throughout the experiment. Other chemicals were also of analytical grade**.**

Chromatographic Conditions

A chromatographic system Agilent 1100 series software consisting of quaternary solvent delivery pump, a degasser, an auto- injector, column oven and photodiode array detector**.** The chromatographic column RP stainless steel column ODS C18 250 x 4.6 x 5 µ L1 packing. The HPLC instrument operated at ambient temperature and the flow rate of mobile phase maintained at 1.0 ml/min. Detection carried at 237 nm and the injection volume was 10 µL .Retention time of Amlodipine Besylate and Atorvastatin Calcium found to be 3.06 minutes 3.913 minutes respectively and the run time was ten minutes.

Mobile Phase

The mobile phase containing (0.03 M) Potassium buffer: Acetonitrile in the ratio of 30:70. pH 2.5 adjusted with ortho-phosphoric acid and solution degassed before use.

Preparation of Standard stock solutions

Blank Preparation: In preparation of a blank solution mobile phase.

Amlodipine Besylate Standard Solution: 10 mg of Amlodipine Besylate tablet accurately weighed and transferred into 10ml volumetric flask. 5 ml of mobile phase transferred in it and agitated thoroughly sonicate for dissolution and diluted with mobile phase up to mark and mixed thoroughly. **(Solution A)**

Amlodipine Besylate Stock Solution: 0.5 ml of **Solution A** transferred into 100 ml volumetric flask and diluted upto the mark by mobile phase.

Atorvastatin Calcium Standard Solution: 10 mg of Atorvastatin Calcium tablet accurately weighed and transferred into 10ml volumetric flask. 5 ml of mobile phase transferred in it and agitated thoroughly sonicate for dissolution and diluted with mobile phase up to mark and mixed thoroughly. **(Solution B)**

Atorvastatin Calcium Standard stock solution:

2 ml of **Solution B** transferred into 100 ml volumetric flask and diluted upto the mark by mobile phase.

Mixture of Standard solution: Mix 0.5 ml of **Solution A** and 2 ml of **Solution B** and diluted by making use of mobile phase in to 100 ml volumetric flask.

Sample solution A 100-mg sample was accurately weighed, transferred in a 50-ml volumetric flask, and dissolved with the diluent (2000 µg mL-1).

Selectivity

The specificity of the method checked for the interference of Retention time of a blank solution (without any sample) and then a drug solution of 20 µg/mL of Atorvastatin Calcium and 5 µg/mL of Amlodipine Besylate injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Atorvastatin Calcium and Amlodipine Besylate. There was no interference of Blank, Atorvastatin Calcium and Amlodipine Besylate in the retention time.

RESULTS AND DISCUSSION

Method Development

The primary target in developing HPLC method was to achieve determination of Amlodipine Besylate and Atorvastatin Calcium in bulk drugs under common conditions that are applicable for the routine quality control of this product in ordinary laboratories. Considering the instability shown by Amlodipine Besylate

and Atorvastatin Calcium in strong acidic and basic media, a mobile phase of water and Acetonitrile with different combination preferred [6-7] and number of stationary phase like C8, C18, CN and NH_2 were employed to achieve it.

In C8 stationary phase using water and acetonitrile the resolution between Amlodipine Besylate and Atorvastatin Calcium achieved but broad peak shape of Amlodipine Besylate and Atorvastatin Calcium obtained having tailing factor about 2.4. In method development different trial experiments were carried to minimize tailing effect NH2 and CN columns (Stationary phase) were also tried and the results obtained were encouraging (tailing factor 2.5) when NH2 used as stationary phase but showed decrease in resolution between Amlodipine Besylate and Atorvastatin Calcium respectively..Similarly CN stationary phase showed improved peak shape of Amlodipine Besylate but eluted at 6.3 and 18.7 respectively for Atorvastatin Calcium [8].

The good resolution and reasonable retention and acceptable for drugs (50 minutes) obtained when high carbon loading, double end capped ODS C18 250 x 4.6 x 5 µ L1 column and mobile phase containing 0.03 M Potassium buffer pH 2.5 with ortho-phosphoric acid and Acetonitrile in the ratio of 30:70 were used . A typical chromatogram with retention time 3.060 and 3.913 for Amlodipine Besylate and Atorvastatin Calcium respectively, which was in good agreement with the earlier reported work [9-10]

Method Validation

System suitability

For System suitability studies, six replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated and are presented in Table 1.

Precision

Repeatability of the method checked by injecting six replicate injections of the solution of Atorvastatin Calcium 20 µg/mL and Amlodipine Besylate 5 µg/mL respectively and the RSD was found to be 1.32 % and 1.73 %. (Table 2). The relative standard deviation of reproducibility and repeatability with respect to peak area and retention time are well within the acceptance criteria. The Resolution between Atorvastatin Calcium and Amlodipine Besylate are 5.56 which is more than 1.5 which is indicative of suitability of the method. [11-13]

Accuracy (Recovery test)

Accuracy of the method tested by carrying out recovery studies at different spiked levels. At each level, (80%, 100% and 120 %.) three determinations were performed. The solutions analyzed, and the percentage recoveries calculated from the calibration curve. The percent recovery and the percent average recovery during our findings are presented in Table 3.

Calibration and linearity

The developed method validated as per ICH guidelines.[1-3] Every 10 µL of the solution of Atorvastatin Calcium and Amlodipine Besylate in the concentration range of 16 to 22 µg/ml for Atorvastatin Calcium and 4 to 6 µg/ml of Amlodipine Besylate injected into the chromatographic system. The chromatograms were developed and the peak area determined for each concentration of the drug solution. Calibration curves of Atorvastatin Calcium and Amlodipine Besylate obtained by plotting the peak area ratio versus the applied concentrations.

Robustness

The robustness of the developed method determined by purposely altering the experimental conditions to evaluate the resolution between Atorvastatin Calcium and Amlodipine Besylate and acid degraded product. The flow rate of the mobile phase was initially 1.0 ml/min; to study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min, the other mobile phase component were held as stated in chromatographic conditions. Similarly effect of temperature on resolution was studied at

25 and 35°C instead of 30°C while the other mobile phase components were held constant stated in chromatographic condition. The results obtained are depicted in Table-4

LOD and LOQ (Sensitivity)

A series of solutions in the range 0.2–1.0% of the related substances concentration (40 µg mL−1) were prepared by dilution of the standard solutions. Each solution (20 µL) was injected five times, the areas were measured for the drug peak, and the standard deviation for the five injections was calculated for each concentration. On the basis of data obtained, the standard deviation at concentration zero calculated and this values used for calculation of the LOD and LOQ. The results are shown in Table-5

Stability of analytical solution

The stability of the standard solutions and the sample solutions tested at intervals of 24, 48 and 72 h. The stability of solutions determined by comparing results of the assay of the freshly prepared standard

solutions. The RSD for the related substances results determined up to 72 h for Atorvastatin Calcium and Amlodipine Besylate and found to be 2.23 and 2.28 % respectively. The related substances values were within ± 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

Table 1: System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Amlodipine Besylate	3.060	1.16	0.96	5700

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Atorvastatin Calcium	3.913	1.21	0.99	6320

Table 2: Linearity and Precision Results.

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Amlodipine Besylate	1.32	80-120	1921.1	0.9974

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Atorvastatin Calcium	1.73	80-120	2634.3	0.9985

Table 3: Recovery Tests for Amlodipine Besylate and Atorvastatin Calcium

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery^
80	50	96.45	95.66
100	100	99.85	100.12
120	150	100.57	100.76

* RSD shown in parenthesis.
^ Average recovery = the average of three levels, nine determinations

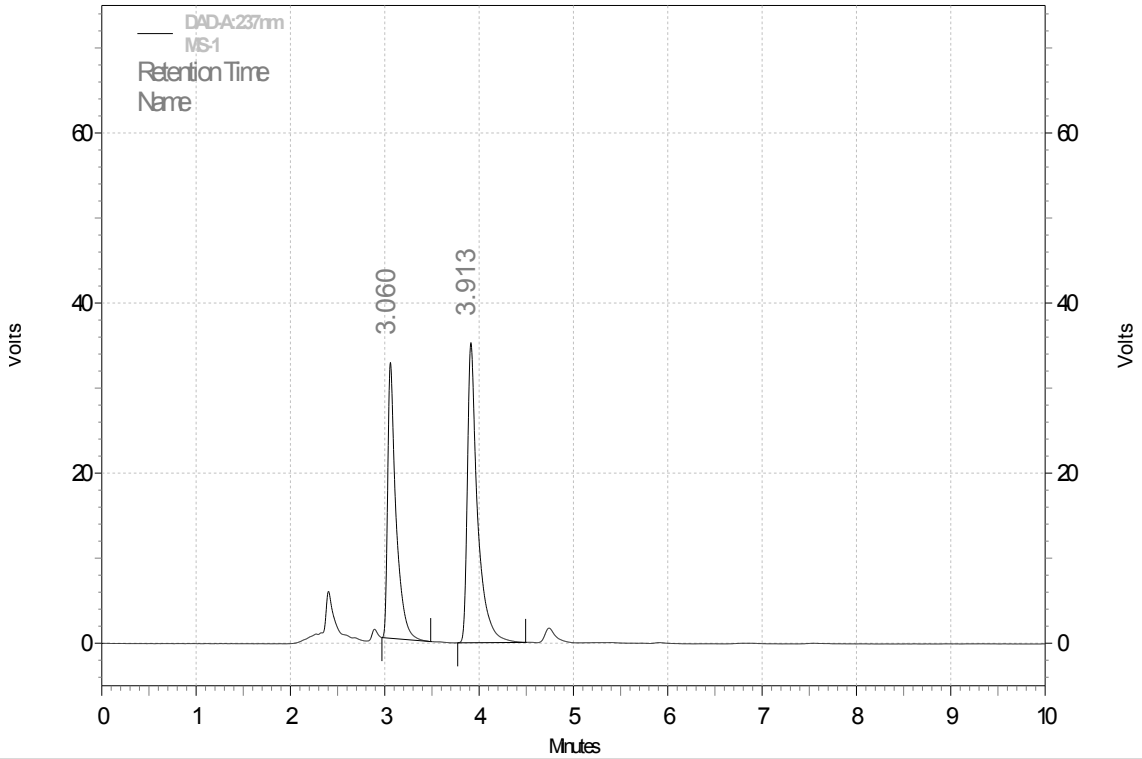
Table 4: Robustness study

Sr. No.	Parameters	Variations	Resolutions between Amlodipine Besylate and Atorvastatin Calcium
1	Temperature	2. at 25 °C	11.23
		3. at 35 °C	9.36
2	Flow rate	1. 0.8 ml/min	10.12
		2. 1.2 ml/min	9.57

Table 5 : LOD and LOQ

Name	%LOD	%LOQ
Amlodipine Besylate	0.10	0.06
Atorvastatin Calcium	0.15	0.09

Figure 3: A chromatogram of the Amlodipine Besylate and Atorvastatin Calcium diluted standard



CONCLUSION

The developed method validated in terms of accuracy, linearity and precision A good linear relationship was observed for Amlodipine Besylate, Atorvastatin Calcium in the concentration ranges of 16–22 µg/mL and 4 to 6 µg/mL respectively. The correlation coefficient for Amlodipine Besylate and Atorvastatin Calcium found to be 0.99 Selectivity experiment showed no interference or overlapping of the peaks either due to diluents with the main peak of Amlodipine Besylate and Atorvastatin Calcium.

The percentage RSD for precision is < 2 which confirms that method is sufficiently precise and the total runtime required for the method is only 10 minutes for eluting both Amlodipine Besylate and Atorvastatin Calcium. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of Amlodipine Besylate and Atorvastatin Calcium.

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