Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(4):35-41

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analytical method development and validation of simultaneous estimation of amlodipine besylate and atorvastatin calcium by RP-HPLC method

K. S. Nataraj*, K. Alekhya, B. Surendra Babu, K. Gnananath and Ch. Bindu

Shri Vishnu College of Pharmacy, Bhimavaram, West Godavari District, Andhra Pradesh, India

---

ABSTRACT

A simple, sensitive and precise RP-HPLC method for simultaneous estimation of Amlodipine Besylate (AMB) and Atorvastatin calcium (AVC) has been developed and validated for determination of compounds in commercial tablet dosage form. The compounds were well separated isocratically on a ODS 3V inertsil column using a mobile phase consisting of buffer (pH 3.5) & Methanol (45:55) with a flow rate of 1.0 ml/min with UV Visible detector at 246nm. Retention time for Amlodipine Besylate and Atorvastatin calcium were found to be 2.19 & 3.71 respectively. The study showed that the reverse phased liquid chromatography is sensitive and selective for detecting Amlodipine Besylate and Atorvastatin calcium using the single mobile phase. The method has been validated for linearity, accuracy and precision. The mean recoveries obtained for Amlodipine Besylate & Atorvastatin calcium were found to be 100.61% and 99.23% respectively. Developed RP-HPLC method was found to be accurate, precise, selective and rapid for simultaneous estimation of Amlodipine Besylate & Atorvastatin calcium in tablets.

Key words: RP-HPLC, Amlodipine Besylate, Atorvastatin calcium

---

INTRODUCTION

Atorvastatin calcium is chemically [R-(R*, R*)]-2-(4-fluorophenyl)-,-dihydroxy-5-(1-methylethyl)-3-phenyl-4 [(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) tri hydrate. It is a competitive inhibitor of HMG-CoA reductase. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, Atorvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.

Amlodipine Besylate is chemically 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine benzene sulfonate Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells.

Few spectrophotometric methods [1],[2], RP-HPLC methods [3],[4],[5] have been reported for simultaneous estimation of AVC and AMB. Literature survey reveals that there is a need for development of economic method for estimation of AVC and AMB. The aim of present study is to develop a new, simple, economic, accurate, efficient reproducible and rugged analytical method for assay of tablet dosage form by RP-HPLC Method and to validate the same as per ICH guidelines.

EXPERIMENTAL SECTION

Apparatus:
A Shimadzu HPLC with LC solution software , UV-Visible Detector (Model SPD 10), Pump (LC 20 AT VP), UV-Visible double beam Spectrophotometer (Nicolet evolution 100), Electronic Balance (Shimadzu), Ultra Sonicator (Citizen, Digital Ultrasonic Cleaner), pH Analyzer (Global digital), Milli Pore (Merck) and HPLC column - Intersil ODS C18(250x4.6 ID) 5µm.

Materials and Reagents:
Water (Milli Q Grade), Sodium dihydrogen ortho phosphate (AR Grade), Methanol(HPLC), Potassium dihydrogen ortho phosphate(AR Grade), Acetonitrile(HPLC Grade), Di potassium hydrogen ortho phosphate(AR Grade),Tri fluoro acetic acid(AR Grade), Ammonium acetate(AR grade), pure drug samples of AVC and AMB are obtained as gift samples from Dr. Reddy's laboratory.

Selection of wavelength by UV-Spectroscopy:
The maximum absorbance of AMB and AVC were found to be 238 and 246 nm respectively from the UV Visible spectrophotometric results. Simultaneous estimation of two spectra shows maximum absorbance at 246nm as shown in the figure (**Fig: 1**)

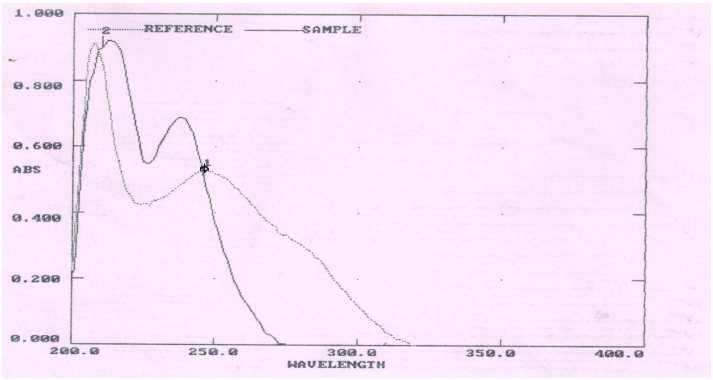

Figure 1: Selection of wavelength by UV-spectroscopy

Preparation of 0.1% Trifluoro acetic acid buffer solution:
0.1ml of Trifluoro acetic acid was diluted to 100mlwith water to give a 0.1% aqueous solution. The pH was adjusted to 3.5 using triethylamine. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of mobile phase:
A mixture of 45 volumes of 0.1% Tri fluoro acetic acid buffer, 55 volumes of Methanol (HPLC grade) was used a mobile phase. The mobile phase was sonicated for 10min to remove gases. It was filtered through 0.45µ nylon filter and degassed.

Preparation of standard solution:
Standard stock solutions of Atorvastatin calcium and Amlodipine besylate (µg/ml) were prepared by dissolving 100 mg of Atorvastatin calcium and 50 mg of Amlodipine besylate in sufficient mobile phase. After that the solution was filtered using 0.45µ syringe filter and sonicated for 5min and diluted to 100 ml with mobile phase. Further dilutions were prepared in 5 replicates by adding 1 ml of stock solution to 10 ml of mobile phase.

Assay sample preparation:
20 tablets (each tablet contains 10 mg of Atorvastatin calcium and 5 mg of Amlodipine besylate) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Atorvastatin calcium and Amlodipine besylate (µg/ml) were prepared by dissolving tablet weight equivalent to 100 mg of Atorvastatin calcium and 50 mg of Amlodipine besylate and dissolved in sufficient mobile phase. After that the solution was filtered using 0.45µ syringe filter and sonicated for 5 min and then diluted to 100 ml with mobile phase. Further dilutions were prepared in 5 replicates by adding 1 ml of stock solution to 10 ml of mobile phase.

Assay of Pharmaceutical formulation:
Separated filtered portions of equal volume of (about 20µl) standard preparation and assay preparations were injected and the chromatogram was recorded and the peak responses of the major peak were measured. The percentage of AMB and AVC in the portion of tablets was calculated by using the following formula.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,
AS: Average peak area due to standard preparation
AT: Average Peak area due to assay preparation
WS: Weight of Amlodipine besylate / Atorvastatin calcium in mg
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
AW: Average weight
P: Standard purity
LC: Label claim

METHOD VALIDATION
The HPLC method was validated in accordance with ICH guidelines.
Precision:
The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision was estimated by repeatability and the repeatability was assessed by analyzing six injection of a homogeneous sample of 50 µg/ml of AMB and 100 µg/ml of AVC.

Accuracy:
The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three different level 80 %, 100 % and 120 % of the target concentration 50µg ml of AMB and 100 µg ml of AVC in triplicate.

Specificity:
The specificity of an analytical method may be defined as the ability to detect the analyte peak in the presence of the byproducts of the analyte or other inactive components such as dosage form, excipients or impurities.

Limit of detection (LOD) and Limit of quantification (LOQ):
Limit of detection and limit of quantification were estimated from signal to noise ratio. LOD is the lowest concentration resulting in a peak area of three times the baseline noise and the equation is LOD = 3.3 x ASD/S. LOQ is the lowest concentration that provides signal to noise ratio more than 10 and the equation is LOQ = 10 x ASD/S, where 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness:
Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. Robustness of the method was carried out by deliberately making small variation in the flow rate, pH of mobile phase, organic phase ratio and column oven temperature by using 400 µg/ml of AMB and 60 µg/ml solution of AVC respectively.

Ruggedness:
Ruggedness was determined between two different labs, different analyst, different instrument and columns. Ruggedness of the method was performed by injecting 50µg/ml of AMB and 100 µg/ml solution of AVC respectively.

RESULTS AND DISCUSSION

Selection of Chromatographic Conditions and Optimization of Mobile Phase:
Mobile phase was optimized to separate AMB and AVC using Intersil ODS, C18 (250x4.6 ID) 5µm. Initially, Phosphate buffer, Citrate buffer and Acetonitrile were tried as a mobile phase in various proportions but the broad peaks for both the drugs were observed. Therefore, we selected 0.1% Tri fluoro acetic acid buffer and Methanol as a mobile phase in 45:55 % v/v ratio. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase was adjusted to 3.5. The flow rate of the mobile phase was 1.8 ml/min. Under optimum chromatographic conditions, the retention time for AMB and AVC was found to be 2.19 and 3.71 respectively when the detection was carried out at 246 nm. A typical chromatogram of two drugs is as shown in figure **(Fig 2)**.

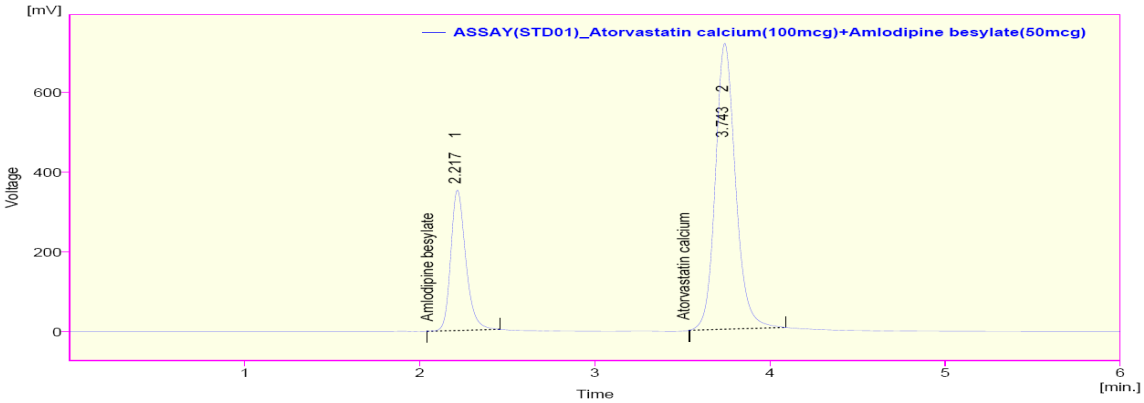

Figure 2: Typical Chromatogram of AMB and AVC

Linearity:
The linearity was determined at five levels over the range of 50 % to 150 % of standard concentration in a diluent and calibration curve constructed by plotting peak area against the respective concentrations. The linearity of AMB and AVC followed in the concentration range of 30 - 70 µg/ml and 60 - 140 µg/ml, respectively. Each sample solution was chromatographed in triplicate and the mean peak area for AMB and AVC calculated. The results are as shown in Table 1 & Calibration plots in figure **(Fig 3, 4)**

Table 1: Linearity studies

PARAMATER	AMB	AVC
Linearity [µg mL-1]	30 – 70	60 – 140
Linearity Equation	Y = 27.935X +850.58	Y = 38.658X +2269
Slope	27.935	38.658
Intercept	850.58	2269
Correlation Coefficient	0.999	0.999

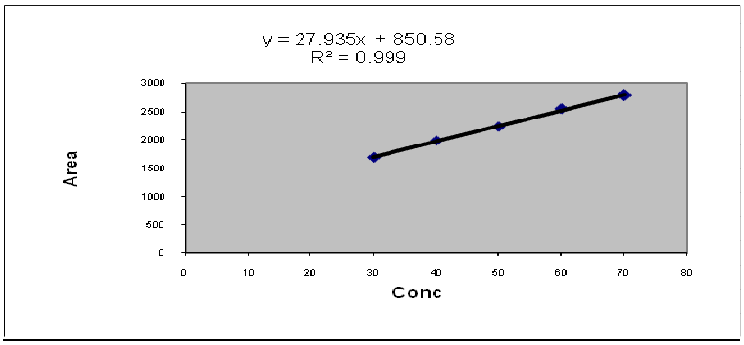

Figure 3: Linearity curve of AMB

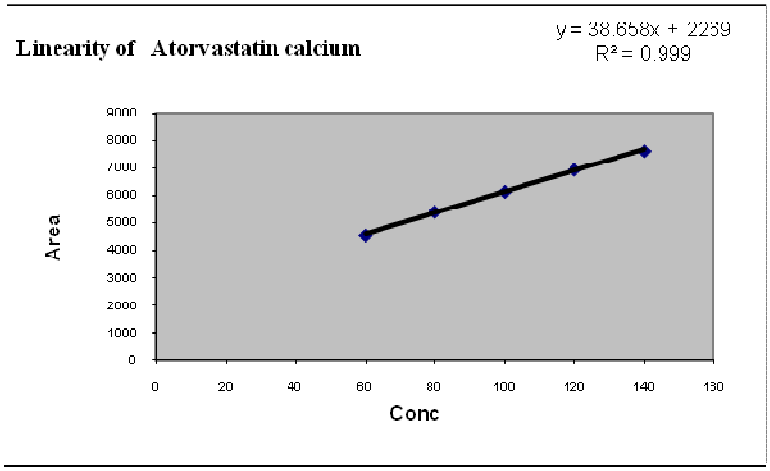

Figure 4: Linearity curve of AVC

Precision:

The precision of this method was evaluated by calculating the % RSD of the peak area of six replicate injections. The RSD for repeatability of AMB and AVC was found to be 0.55 and 1.27 %, respectively. The RSD values for intra-day precision for AMB and AVC was found to be in the range of 0.30-0.90 and 0.57 -0.95 %, respectively. The assay method precision acceptance criteria set in the validation was RSD ≤ 2.0%. Results of precision study are shown in Table 2.

Table 2: Precision Intraday studies

	Amlodipine besylate		Atorvastatin calcium	
	%RSD (Rt)	%RSD(Area)	%RSD (Rt)	%RSD(Area)
Day 1	0.25	0.65	0.13	0.99
Day2	0.27	0.30	0.15	0.57
Day3	0.09	0.90	0.10	0.95

Specificity and Selectivity:

The specificity of the method was checked for the interference of retention time of tablet formulation and standard drug under optimized condition. Specificity of the method was determined by comparing the chromatogram obtained from tablet formulation and standard drug. As there was no interference of excipients and the retention time of standard drugs and tablet formulation were same, so method was specific and selective. A typical chromatogram of specificity of two drugs is as shown in figure (**Fig 5).**

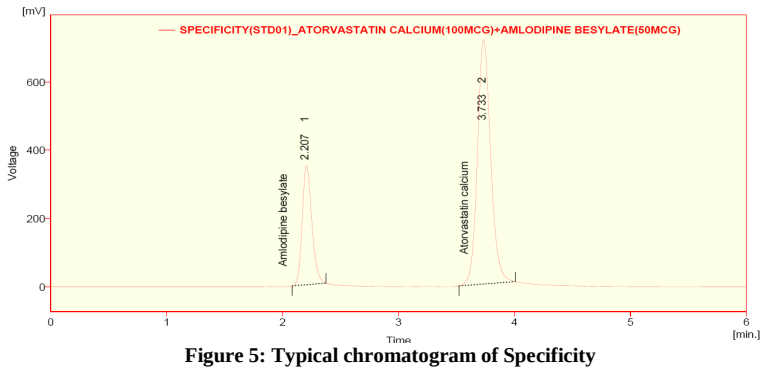

Figure 5: Typical chromatogram of Specificity

Accuracy:

Accuracy was evaluated as percentage relative error between the found mean concentration and added concentration for AMB and AVC. The result obtained for three different concentration levels i.e. 80 %, 100 % and 120 % showed

acceptable % recoveries in the range of 100.33% - 100.24 % for AVC and 99.25% - 99.68 % for AMB which suggests that accuracy is excellent for both drugs. The results are shown in Table 3.

Table 3: Recovery studies of AMB and AVC

Drugs	Amount of drug added (%)	% Recovery	% Average recovery
AMB	80	99.68	99.53%
	100	99.25	
	120	99.68	
AVC	80	100.33	100.61%
	100	101.27	
	120	100.24	

Sensitivity:
The limit of detection (LOD) and limit of quantification (LOQ) for AMB and AVC was found to be 1.868 and 5.611 µg and 2.700 and 8.182 µg, respectively. The low values of LOD and LOQ indicates high sensitivity of the method.

Robustness and Ruggedness study:
The Robustness of the method evaluated by changing the chromatographic condition and results were examined. Ruggedness test was determined between two different analyst and instrument under same environmental condition. The percentage RSD was below 2.0%, Showed robustness and ruggedness of method. The results were shown in Table 4 & Table 5.

Table 4: Results for Ruggedness

Atorvastatin calcium	%Assay	Amlodipine besylate	%Assay
Analyst 01	101.21	Analyst 01	100.54
Analyst 02	99.98	Analyst 02	99.83
%RSD	0.86%	%RSD	0.50%

Table 5: Results of Robustness study

Parameter	Amlodipine besylate		Atorvastatin calcium	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate 1.2ml/min	1.780	1.3684	2.980	1.185
1ml/min	2.930	1.444	4.907	1.308
0.8ml/min	2.980	1.185	3.660	1.450
Wavelength 244nm	2.223	1.364	3.710	1.219
246nm	2.203	1.409	3.727	1.281
248nm	2.203	1.409	3.707	1.219

System Suitability:
Tested according to USP 2009, system suitability tests were an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. System suitability tests were used to verify that the resolution and reproducibility were adequate for the performed tests. The parameter of these tests as column efficiency (number of theoretical plate), tailing factor, resolution, peak asymmetry and capacity factor were calculated for standard solutions. The results obtained from validation of the methods and system suitability studies are summarized in Table 6.

Table 6: Results for system suitability

PARAMETER		AMB	AVC
1	Retention time	2.200	3.731
2	Theoretical plates	3125	5116
3	Asymmetry	1.40	1.224

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Atorvastatin calcium and Amlodipine besylate was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

REFERENCES

[1] MR Khan; Deepti Jain, *Indian J Pharm Sci*., **2006**, 68(4), 546-548.
[2] R Sahu; Vandana B Patel, *Indian J Pharm Sci*., **2007**, 69(1), 110-111.
[3] D A Shah; KK Bhatt; M B Shankar; R S Mehta; T R Gandhi; S L Baldania, *Indian J Pharm Sci*., **2006**, 68(6), 796-799.
[4] D. A. Shah; K. K. Bhatt; R. S. Mehta; S. L. Baldania; T. R. Gandhi, *Indian J Pharm Sci.*, **2008**, 70(6), 754–760.
[5] A Mohammadi; N Rezanour; M Ansari Dogaheh; F Ghorbani Bidkorbeh; M Hashem; RB Walker, *J Chromatogr B Analyt Technol Biomed Life Sci;* **2007**, 846(1-2), 215–221.