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International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 695-698, April-June 2011

Simultaneous determination of Amlodipine besylate and Atorvastatin calcium in Pharmaceutical tablet formulation by High Performance Thin Layer Chromatographic method

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Abstract : Simple, rapid, accurate, precise, reliable and economical high performance thin layer chromatography method have been proposed for simultaneous determination of amlodipine besylate and atorvastatine calcium in pure and commercial formulation without any prior separation or purification. Normal phase thin layer chromatography plate (silica gel 60 F_{254}) was used as stationary phase chloroform-methanol-acetic acid 85:10:5 (v/v) as mobile phase to determine two pharmaceutically active ingredients.This system gave a good resolution for amlodipine besylate (R_f value of 0.27 ± 0.015) and atorvastatin calcium (R_f value of 0.52 ± 0.015). Determination was by densitometry in the absorbance mode at 247 nm. The method was found to be linear in the range of 100-800 ng/ spot for amlodipine besylate and atorvastatine calcium. The method was validated for precision and recovery. The LOD value for amlodipine besylate and atorvastatine calcium were found to be 60 ng/ spot and 40 ng/ spot respectively and the LOQ value was found were found to be 100 ng/ spot for both the drug respectively. The result of study showed that the proposed high performance liquid chromatographic method is useful for the routine determination of amlodipine besylate and atorvastatin calcium in tablet dosage form.

Keywords: High performance thin layer chromatography; Amlodipine besylate; Atorvastatin calcium.

Introduction

Amlodipine besylate (AMLB), 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(o-chlorophenyl)-1,4-di hydro-6-methyl-3,5-pyridinedicarboxylate, mono benzenesulphonate, is a calcium channel blocker. It inhibits the trans-membrane influx of calcium ions into vascular smooth muscles and cardiac muscle. Atorvastatin calcium (ATVC) is chemically [R-(R*,R*)]-2-(4-flurophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt trihydrate. 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, and primary dysbetalipoproteinemia. The literature survey reveals many analytical mehthods for the quantitative determination of either AMLB or ATVC alone [1], [2], [3]. Some spectrophotometric and chromatographic methods have also been developed for simultaneous determination of AMLB and ATVC [4], [5], [6], [7], [8]. However, no method has been reported till date for the determination of AMLB and ATVC by high performance thin layer chromatographic method (HPTLC). So, in this

communication an HPTLC method is proposed for simultaneous determination of AMLB and ATVC.

Experimental

Instrumentations and conditions

Chromatography was performed on 10 cm x 10 cm aluminum plates precoated with 250-µm layer of silica gel 60 F 254 (E.Merck, Dar mstadt, Germany). The plates were prewashed with methylene chloride and methanol (1:1) and activated at 70 ^{0}C for 20 min. Sample was applied to the plates as bands 6 mm wide 15 mm apart and 8 mm from the bottom of the plate by means of a camag (Switzerland) Linomate 5 sample applicator equipped with a 100 µl syringe (Hamilton, Bonaduz, Switzerland).

Linear ascending development was performed in a 20 cm x 10 cm twin through glass chamber (Camag), with chloroform-methanol-acetic acid 85:10:5 (v/v) as mobile phase, after saturation of the chamber with mobile phase vapor for 5 min. The development distance was 7 cm and the development time approximately 20 min. The plates were then air dried and densitometric scanning was performed with a camang TLC scanner 3 at 247 nm for all measurements. The scanner was operated by Wincats software Version 1.4.2. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimensions were 5 mm x 0.45 mm and the scanning speed was 20 mm S^{-1}.

Reagents

Methanol, chloroform and methylene chloride (AR grade) were obtained from Allied Chemical Corporation (Vadodara, India). Standard AMLB and ATVC were provided as a gift sample by Torrent Pharmaceuticals Ltd. (Ahmedabad, India) and M/s. Alembic Ltd (Vadodara, India) respectively. Avas-AM tablets with 5 mg AMLB and 10 mg ATVC per tablet were procured from local market.

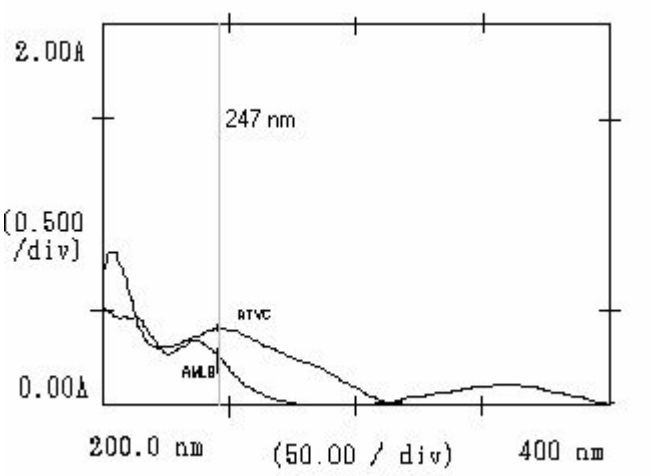
Selection of wavelength for detection

10 µg ml^{-1} solutions of AMLB and ATVC were scanned in the range of 200 to 300 nm by use of Shiamadzu1601 UV-visible spectrophotometer. The wavelength of maximum absorbance of ATVC (247 nm) was selected as analytical wavelength as at this wavelength AMLB also have significant absorbance (Fig 1).

Procedure

All reagents were tested for stability in solution and during the actual analysis the behaviour of the analyte remained unchanged up to about 24 hours from their preparation at the room temperature. Both the drugs were found to be stable during each kind of experimental measurements. Each measurement was done at room temperature.

Figure 1 Overlay spectra of AMLB and ATVC



Preparation of calibration curve

The standard stock solution of AMLB (0.1 g L^{-1}) and ATVC (0.1 g L^{-1}) in methanol were applied on the top of each other on a TLC plate, in the range 1- 8 µl, by use of the Linomate 5 sample applicator and 100 µl syringe. The plate was developed and scanned. This was repeated five times and peak areas were recorded. Calibration curves of peak area against respective concentrations were established separately for AMLB and ATVC.

Method Validation

The proposed method was validated in terms of linearity, precision, accuracy, LOD and LOQ.

Analysis of Tablet Formulation

Twenty tablets were weighted accurately and ground to fine powder. A quantity of powder equivalent to 5 mg AMLB and 10 mg ATVC was weighed and transferred to 10 ml volumetric flask containing approximately 5 ml methanol. The mixture was ultrasonicated for 5 min and then diluted to volume with methanol. The solution was filtered using Whatman no. 41 paper and 4 µl of the filtrate was applied to a TLC plate to furnish 200 ng band for AMLB and 400 ng band for ATVC. After chromatographic development, the peak areas of the bands were measured at 247 nm and the amount of each drug in tablet was determined from the respective calibration curve. The procedure was repeated six times for the homogenous powder sample.

Figure 2 Chromatogram showing R_F values of AMLB and ATVC

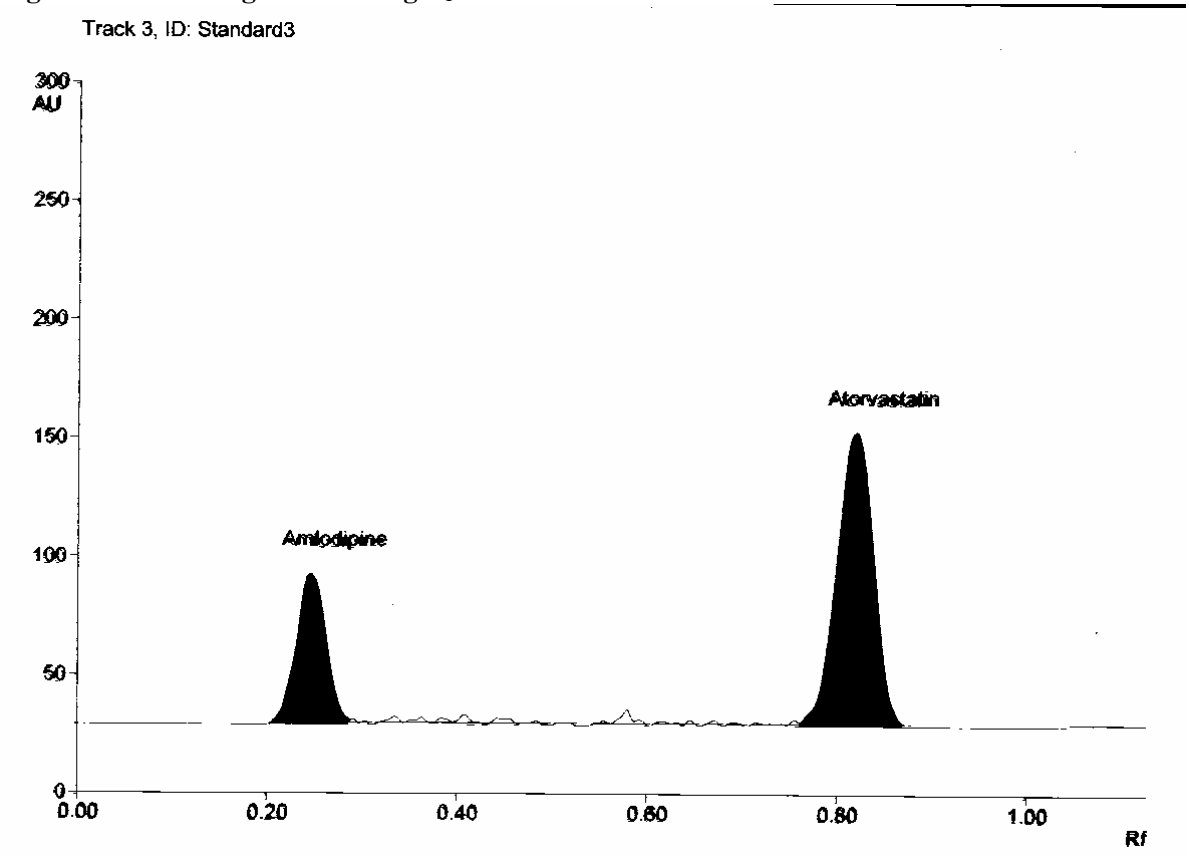


Table 1 Results of recovery study of AMLB and ATVC

% Amount added to pre-analyzed sample	*% Recovery ± SD for method	
	AMLB	ATVC
50%	102.43 ± 1.014	100.42 ± 1.990
100%	99.88 ± 1.988	100.92 ± 1.433
150%	101.19 ± 0.593	100.81 ± 1.172
Mean recovery	101.1667 ± 1.198	100.7167 ± 1.53

*Mean of five determinations

Results

HPTLC method is developed for the simultaneous determination of AMLB and ATVC in their combined dosage form.

Analytical estimation was carried out at 247 nm. Chloroform-methanol-acetic acid (85:15:5 v/v) was selected as a mobile phase. The developed method obeys Beer's law in the range of 100 to 800 ng / spot. The method was validated in terms of accuracy, precision, LOD and LOQ .

Accuracy of the method was confirmed by performing recovery studies. Recovery experiments were carried out by the standard addition method at three different levels 50,100 and 150 % by addition of known amount of AMLB and ATVC to a pre-analyzed sample of commercial tablets. The experiment was repeated five times and the amount of standard recovered was calculated in terms of mean recovery with the upper and lower limits of percentage standard deviation. The results of accuracy are shown in Table 1.

Intra-day precision and inter-day precision for the developed methods were measured in terms of % RSD. The experiments were repeated five times a day for intra-day precision and on five different days for inter-day precision. The mean of % R.S.D (% R.S.D= [S/X] 100, where S is standard deviation and X is mean of the sample analyzed) for intra-day precision was found to be 1.821 for AMLB and 1.456 for ATVC and that for inter-day precision was 1.432 and 1.231 respectively.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated according to 3 s/m and 10 s/m criterions, respectively, where s is the standard

deviation of the absorbance (n=10) of the sample and m is the slope of the corresponding calibration curve. LOD values were found to be 60 and 40 ng/spot for AMLB and ATVC respectively and LOQ values were 100 ng/ spot for both the drugs. Assay results of AMLB and ATVC in combined commercial tablet formulation were found to be 98.216 ± 1.23 for AMLB and 99.022 ± 1.87 for ATVC.

Discussion

The densitometric estimation was performed at analytical wavelength of 247 nm as both the drugs showed significant absorbance at this wavelength (Fig. 1).

Different mobile phases containing cyclohexane, Isopropyl Alcohol, Chloroform, Methanol, Acetic acid in different proportions were examined. As Chloroform-methanol-acetic acid (85:15:5 v/v) resulted in acceptable resolution of the bands with R_F values of 0.27 ± 0.015 for AMLB and 0.52 ± 0.015 for ATVC, it was finally selected as a mobile phase. The densitogram obtained from a mixed standard solution of AMLB and ATVC shown in Fig.2 shows the well resolved peaks of AMLB and ATVC..

Beer's law range of 100 to 800 ng / spot for both the drugs revealed the sensitivity of the method. The regression equation Y = 3.400 + 3.400 * X with correlation coefficient 0.99946 for AMLB and Y = -124.435 + 8.661 * X with correlation coefficient (r) 0.99865 for ATVC clearly indicates the satisfactory results. The mean recovery of 100-101 % for both the drugs proved the accuracy of the proposed method and the % RSD of < 2 proved the precision of the method. The assay results of tablet dosage form reveal that the method could be successfully extrapolated to the simultaneous estimation of AMLB and ATVC in combined dosage form without prior separation of individual drugs.

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