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Development and validation of a high performance thin layer chromatographic method for the simultaneous estimation of Atorvastatin calcium and Amlodipine besylate as the bulk drugs and in the tablet dosage form

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ABSTRACT

A simple,accurate, reproducible and economical high performance thin layer chromatographic method (HPTLC) has been developed and validated for the simultaneous estimation of Atorvastatin Calcium (ATV) and Amlodipine Besylate (AML) without previous separation. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of toluene: methanol: triethylamine in the ratio 3:7:0.2 (v/v/v). The detection of the spots were carried out at 253 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 10 to 50 ng/spot for both the drugs. The limit of detection and limit of quantitation obtained by this method were 2ng/ spot and 10 ng/ spot for Atorvastatin and 5ng/ spot and 10 ng/ spot for Amlodipine respectively. Statistical analysis proves that the proposed method can be successfully used to determine the drug content of marketed formulation.

INTRODUCTION

Atorvastatin calcium, 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 (2:1) trihydrate, is an inhibitor of HMGCoA reductase, an enzyme involved in cholesterol biosynthesis[1,2].Amlodipine besylate ,a dihydropyridine calcium channel blocker , is approved for the treatment of angina pectoris and is chemically2-[(2-Aminoethoxy) methyl] -4-(2-chlorophenyl)-3ethoxy carbonyl-5- methoxycarbonyl-6-methyl-1,4-dihydropyridine benzene sulfonate[3]. The fixed-dose combination containing Atorvastatin Calcium and Amlodipine Besylate improved the fibrinolytic balance more than either single agent in hypertensive, hypercholesterolemic patients with insulin resistance and could potentially improve medication compliance. The survey of literature for ATV revealed that HPLC[4,5], GC-MS[6],LC-MS[7] HPLC-Electron spray tandem mass spectrometry[8] and HPTLC [9] have been reported for its estimation. HPLC method is official in IP[10] for the estimation of ATV while the assay methods for the determination of AML is official in IP[11], BP[12], EP[13] and USP[14], but these methods do not involve simultaneous determination of ATV and AML. Detailed survey of literature for AML revealed several methods based on different techniques, viz. spectrophotometric methods[15-17] , HPTLC methods[18,19], HPLC methods [20,21] and adsorptive square wave anodic stripping voltammetry [22] , spectrophotometry[23] and HPLC[24] for simultaneous determination of ATV and AML. In the present investigation, an accurate and precise HPTLC method has been developed for the simultaneous estimation of Atorvastatin Calcium and Amlodipine Besylate in combined dosage forms.The reported HPTLC method used chloroform-methanol-acetic acid 85:10:5 (v/v) as mobile phase, *the International Programme on chemical safety environmental health criteria*[25] has recommended that chloroform has exposure risks with a residence time of several months in the atmosphere and is removed from the atmosphere through chemical transformation . The proposed method excludes the use of chloroform and there by reduces environmental pollution.

MATERIALS AND METHODS

Reagents and Chemicals:

The reference standard of Amlodipine Besylate and Atorvastatin Calcium were gift samples from Cadila Healthcare Ltd. and Intas Pharmacetical Industry respectively.All other chemicals used were analytical grade obtained from SD fine

chemicals.

Instrumentation

The samples were spotted in the form of bands of width 6mm with a Camag 100 µl sample syringe (Hamilton) on silica gel precoated aluminum plate 60 F_{254}, (20 cm×20 cm with 200 µm thickness; E. Merck) using a Camag Linomat V sample applicator. The plates were pre-washed by methanol and activated at 110 °C for 5 min. prior to chromatography. The space between two bands was kept at 10 mm. The slit dimension was kept at 4 x 3 mm and 4.0 mm/s scanning speed was employed. The monochromator bandwidth was set at 10 nm, each track was scanned thrice and baseline correction was made. The mobile phase consisted of toluene: methanol: triethylamine in the ratio 3:7:0.2 (v/v/v). Linear ascending development was carried out in a Camag twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature (25 °C±2) at relative humidity of 55%±5. Subsequent to the development; TLC plates were dried with the help of air dryer. Densitometric scanning was performed on Camag TLC scanner II in the absorbance-reflectance mode at 254 nm using CATS software. The concentrations of the compound chromatographed were determined using densitogram area.

Preparation of standard solution

Standard stock solutions of Atorvastatin Calcium and Amlodipine Besylate were prepared by dissolving 25mg of each drug separately in two standard flasks and the final volume was made up to 25ml with methanol to get a concentration of 1mg/ml. The above solutions were diluted with methanol to get concentration of 100µg/ml each. Different volumes of the above solutions, viz. 0.5, 1.0, 1.5,2.0, 2.5, and 3 µl were pipette out into two sets of six 10ml standard flasks and the final volume was made up with methanol to get concentrations of 5µg/ml- 30µg/ml which were equivalent to 5ng/µl- 30 ng/µl for application purpose to be spotted on to the plate followed by development and scanning.

Preparation of sample solution

The sample of powdered tablets equivalent to 10mg of ATV and 5mg of AML (two brands) [equivalent to one tablet] were weighed and transferred to 10 ml volumetric flask. For analysis of drug Amlodipine Besylate, a standard addition method was used. An accurately weighed 5 mg of pure AML was added to the accurately weighed samples in the volumetric flasks to bring the ratio of AML and ATV to 1:1. The complete extraction of the drug was achieved by sonicating the flasks for 30min. at room temperature (25±2^{0}C). The above solution was diluted to get a concentration of 100µg/ml. From the above stock solutions, 1.5µl was diluted to get drug concentrations of 30ng /spot for each and spotted on to the plate. The chromatographic plate was then developed in a pre-saturated twin trough chamber containing mobile phase. After development, the bands of the drugs were scanned at 253 nm using a densitometer. The peak area of standard and samples were used to calculate the amount of ATV and AML present per tablet.

Validation of the method

The method was validated for linearity, limits of detection ,limit of quantitation intraday and inter day precision, robustness, specificity, ruggedness and accuracy. The limits of detection and limit of quantitation were calculated from the slope (S) of the calibration plot and the standard deviation (SD) of the response . The ruggedness and precision studies of the method were estimated by performing six determinations of the drug solutions at two different concentrations namely 20 and 40ng /spot .The robustness was checked by the analysis of the sample solutions after making small changes to mobile phase composition. Recovery experiments were carried out by the standard addition method at three different levels 20, 40, and 60% by the addition of known amount of ATV and AML to a pre-analyzed sample of commercial tablets. The experiment was repeated three times and the amount of standard recovered was calculated in terms of mean recovery.

RESULTS

HPTLC method is developed for the simultaneous determination of ATV and AML in their combined dosage form. Analytical estimations was carried out at 253nm. Toluene:

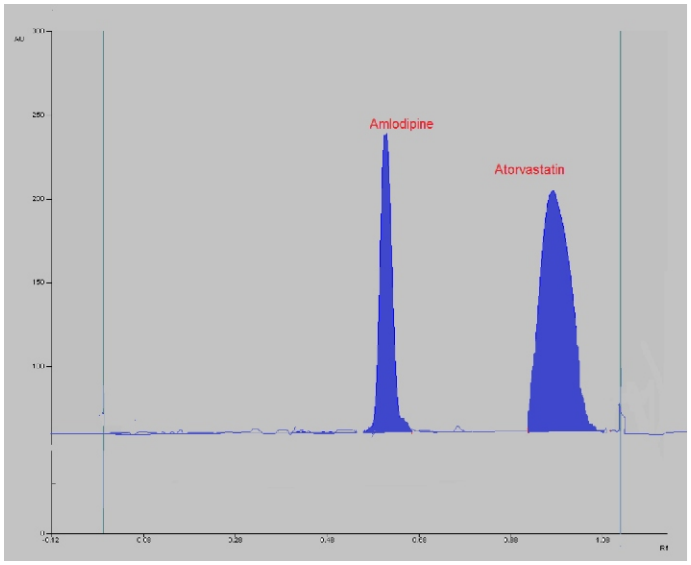

Fig. 1. Chromatogram of Atorvastatin & Amlodipine

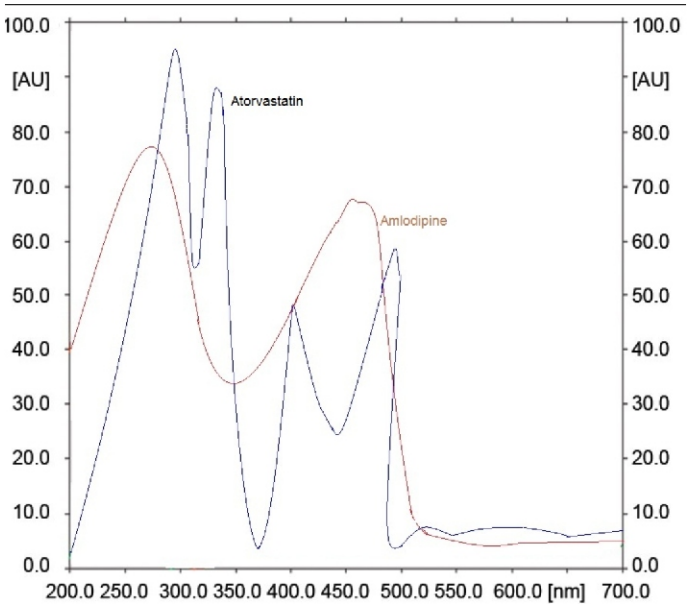

Fig. 2. Overlain absorption spectrum of Atorvastatin & Amlodipine

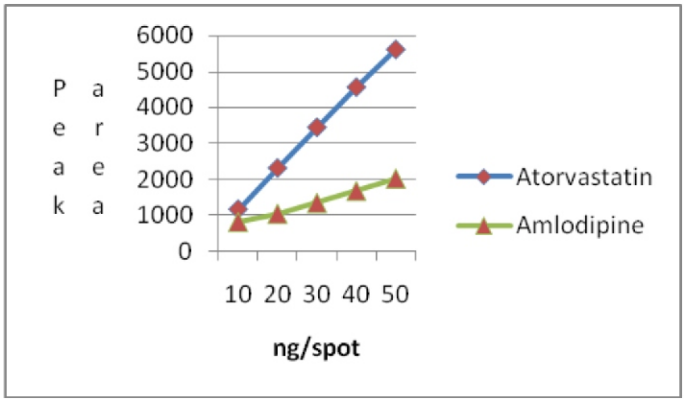

Fig. 3. Overlain calibration plot of Atorvastatin & Amlodipine

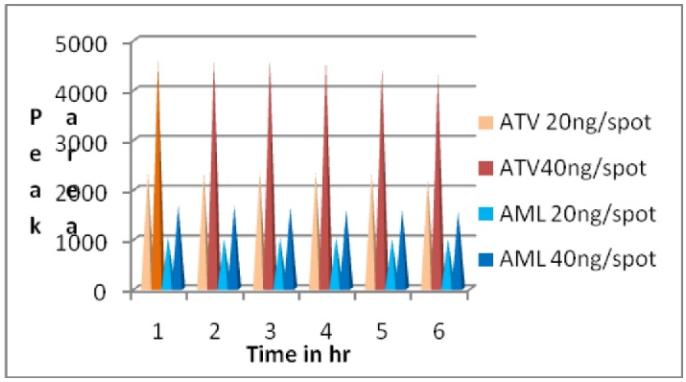

Fig. 4. Stability profile of the developed spots by HPTLC

methanol : triethylamine in the ratio 3:7:0.2 (v/v/v) was selected as a mobile phase. With this solvent system,sharp and well defined peaks for Atorvastatin and Amlodipine were resulted with R_f values 0.88 ± 0.02 and 0.55 ± 0.02 Atorvastatin and Amlodipine respectively (Fig .1) . The overlain absorption spectrum of Atorvastatin and Amlodipine is given in Fig .2. The developed method obeys Beer's law in the range of 10-50 ng/spot for both the drugs and the overlain calibration plots are shown in Fig.3.The optical characteristics are listed in Table.1. The method was validated in terms of limits of detection,limit of quantitation, intraday and inter day precision, robustness, ruggedness and accuracy.. The limit of detection and limit of quantitation obtained by this method were 2ng/spot and 10 ng/spot for Atorvastatin and 5ng/spot and 10 ng/spot for Amlodipine respectively. The results of intraday precision and interday precision studies are shown in Table .2. The results of ruggedness studies are listed in Table.3. The stability profile of the developed spots are shown in Fig: 4. The results of analysis of the combination tablets (two brands) were furnished in Table.4 and the corresponding chromatogram were shown in Fig: 5 & Fig: 6. The results of recovery studies are shown in Table 5.

DISCUSSION

Different mobile phase compositions were tried to optimize

Table 1. Optical parameters for calibration plot

Parameter	Observed Value	
	ATV	AML
Linearity range	10-50ng/spot	10-50ng/spot
Correlation coefficient	0.9998	0.99724
Regression line equation:	y=149.45+109.539x	y=465.93+30.283x
Slope	109.539	30.283
Intercept	149.45	465.93

Table 2. Inter day and intra day precision data

Amount (ng/spot)		Intraday precision		Interday precision	
		Mean area(AU)	RSD (%)	Mean area(AU)	RSD (%)
ATV	20	2358.2	0.98%	2348.6	1.21%
	40	4572.8	0.55%	4598.9	1.09%
AML	20	1031.6	0.38%	1039.5	1.07%
	40	1685.4	0.59%	1753.4	1.21%

[a] n = 6

Table 3. Data showing ruggedness of HPTLC method

DRUG	Variable	Recovery(%)[a]	RSD(%)[b]
ATV	Analyst I	99.99	1.10
	Analyst II	100.65	1.05
AML	Analyst I	100.05	1.19
	Analyst II	100.75	1.15

[a] n = 6 [b] Average for two amounts: 20 and 40ng/band.

Table 4. Report of Analysis of combination tablets

Brand name	Label claim (mg/tablet)	Estimated		/ t [b] / test	F [b] test
		mg/tablet [a]	%label Claim		
Caduet	ATV 10mg	9.92±0.056	99.20	1.48	4.48
	AML 5mg	4.99±0.089	99.8	1.98	
Zivast	ATV 10mg	9.96±0.098	99.60	1.38	4.44
	AML 5mg	4.89±0.039	97.08	1.68	

[a] Mean ± SD, n=6

[b] Tabulated t-value is 2.77 and tabulated F-value is 6.39.

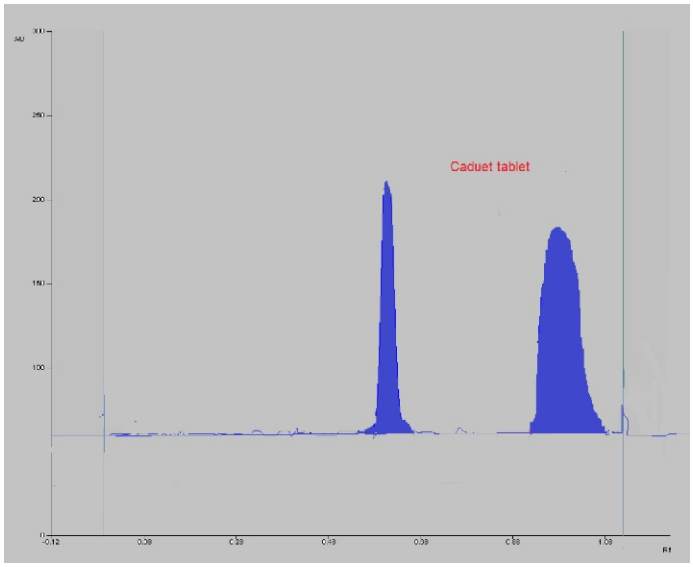

Fig. 5. Chromatogram of Caduet tablet 30 ng/spot

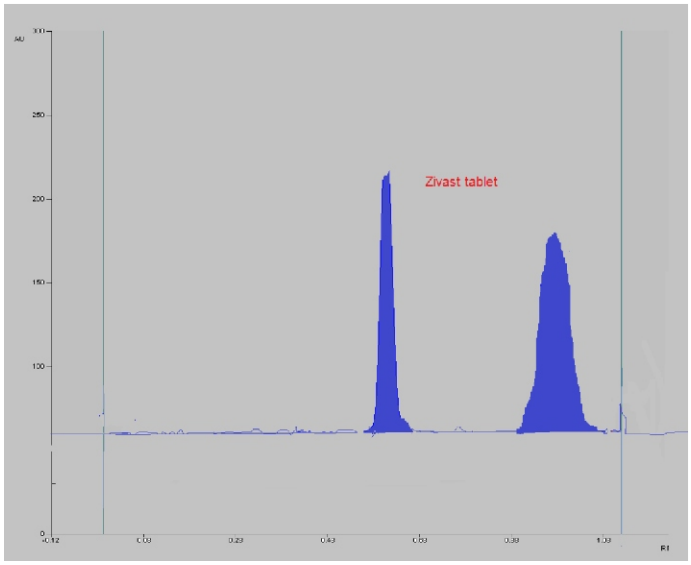

Fig. 6. Chromatogram of Zivast tablet 30 ng/spot

Table 5. Results of recovery studies

Drug	Amount of drug added(%)	Total amount (ng)*	Amount recovered (ng)	Recovery (%)	RSD (%)
ATV	20	36	36.28	100.80	1.07
	40	42	42.56	101.34	1.47
	60	48	47.71	99.34	1.34
AML	20	36	36.32	100.90	1.05
	40	42	42.77	101.84	1.40
	60	48	47.71	99.44	1.42

the chromatographic conditions. Initially,toluene: methanol: glacial acetic acid (2:7:1v/v/v) was used. The best results were obtained by the use of toluene:methanol :triethylamine in the ratio 3:7:0.2 (v/v/v). With this solvent system, sharp and well defined peaks for Atorvastatin and Amlodipine were resulted with R_f values 0.88 ± 0.02 and 0.55 ± 0.02 for Atorvastatin and Amlodipine respectively.The linear regression data of calibration plot showed a good linear relationship over a concentration range of 10-50 ng/spot for both the drugs which shows the adequate sensitivity of the method.The regression equation Y = 149.45 + 109.539X with correlation coefficient (r) 0.9998for ATV and Y = 465.93 + 30.283 X with correlation coefficient (r) 0.99724 for AML clearly indicates the satisfactory results. The mean recovery of 99.34% - 101.34% for Atorvastatin and 99.44% -101.84% for Amlodipine proved the accuracy of the proposed method and the low values of % RSD proved the precision of the method.

CONCLUSION

By observing the validation parameters, the method is found to be simple, sensitive, accurate and precise. Hence the proposed method can be employed for the routine analysis for the simultaneous estimation of Atorvastatin and Amlodipine in bulk and combined dosage form.

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