Eurasian Journal of Analytical Chemistry, 2016, **11**(3), 155-168

Development and Validation of HPTLC Method for the Simultaneous Estimation of Amlodipine Besylate and Atorvastatin Calcium in Combined Dosage Form

Nagasarapu Mallıkarjuna Rao
Department of Pharmaceutical Analysis, College of Pharmacy, INDIA.
D.Gowri Sankar
Department of Pharmaceutical Analysis, A.U college of Pharmacy, Andhra University, INDIA.

•*Received 24 September 2015* •*Revised 29 January 2015* •*Accepted 03 February 2016*

This present study reports for the first time simultaneous quantitation of Amlodipine besylate and Atorvastatin calcium by HPTLC from a combined dosage form. Chromatographic separation of the drugs were performed on aluminum plates precoated with silica gel 60 F_{254} used as stationary phase and the chromatogram was developed using Ethyl acetate: Methanol: Ammonia (7.5 : 2 : 0.5 %v/v/v) as mobile phase. Amlodipine besylate and Atorvastatin calcium showed Rf values 0.50 ± 0.02 and 0.26 ± 0.02 respectively. Densiometric analysis of both the drugs was carried out in the absorbance mode at 365 nm. The method has been successfully applied to tablets and was validated according to ICH Harmonized Tripartite guidelines. The linearity regression analysis for calibration showed 0.9983 (r^2) and 0.9994 (r^2) for amlodipine besylate and atorvastatin calcium with respect to peak area and height in the concentration range of 100-500ng/spot and 200-600ng/spot respectively. The percentage recovery for amlodipine besylate was found to be 101.82 (at 50%), 99.12 (at 100%) , 100.5(at 150%) and 101.41 (at 50%), 101.71 (at 100%), 99.5(at 150%) for atorvastatin calcium. The limit of detection was 30 ng/spot and 60 ng/spot for amlodipine besylate and atorvastatin calcium respectively. The limit of quantification was found to be 100 ng/spot and 200 ng/spot for amlodipine besylate and atorvastatin calcium respectively. The developed TLC technique is precise, specific and accurate. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use.

Keywords: amlodipine besylate and atorvastatin calcium, HPTLC; simultaneous estimation, quantitative analysis

Correspondence: Nagasarapu Mallıkarjuna Rao
Research scholar of Jawaharlal Nehru Technological University, Department of Pharmaceutical Analysis, College of Pharmacy, Kakinada, Andhra Pradesh, INDIA
E-mail: mallimpharmmba@gmail.com
doi: 10.12973/ejac.2016.129a

ISSN: 1306-3057

INTRODUCTION

Amlodipine is a white crystalline powder which is slightly soluble in water, sparingly soluble in ethanol and freely soluble in methanol. It is official in B.P. Chemically Amlodipine, (Figure 1.) is 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4- dihydro-6- methyl-3, 5-pyridine dicarboxylate-benzenesulfonat [1]. Amlodipine is a dihydropyridine derivative with calcium antagonist activity [2]. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina [3]. Amlodipine acts by inhibiting the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. Atorvastatin is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent [4].

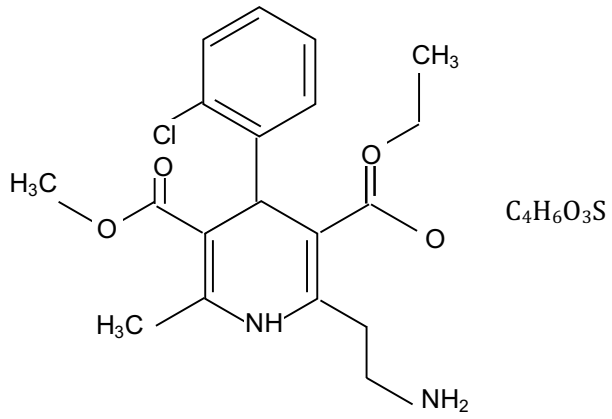

Figure 1. Amlodipine besylate

Chemically, Atorvastatin (Figure 2) is [R-(R*, R*)]-2-(4-flurophenyl)-B, B-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid [5]. Atorvastatin is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylgultaryl-coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of LDL receptors on the surface of the cells, which inturn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood [6-8].

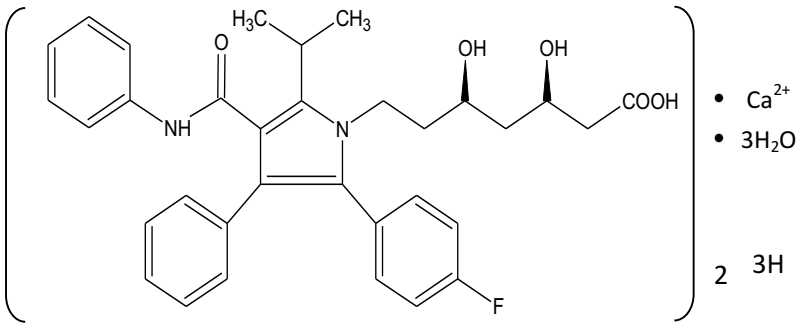

Figure 2. Structure of atorvastatin calcium

HPLC methods are official in I.P [9] for the estimation of atorvastatin while in I.P [10], B.P [11], E.P[12] and USP [13] for the determination of amlodipine, but they do not involve simultaneous determination of atorvastatin and amlodipine. Detailed survey of literature for atorvastatin revealed several methods based on different techniques, viz. HPLC [14-16] and LC-MS [17-19] for its determination in plasma/serum; HPLC [20] for its determination in human serum and pharmaceutical formulations; HPLC [21-22]; Similarly, survey of literature for amlodipine revealed methods based on spectrophotometry [23], RP-HPLC [24] using fluorescence detection, HPLC-tandem mass spectrometry [25-26], RP-HPLC using UV detection [27-28], HPLC [29-33] in combination with other drugs, Flow injection analysis using UV-detection [34], stability indicating HPLC [35] and stability indicating HPLC [36] in combination with benazepril hydrochloride have been reported. Spectrophotometric [37], HPLC [38-39] methods have been reported for simultaneous determination of atorvastatin and amlodipine. The reported HPLC methods involve costly sophisticated instrumentation and time consuming process. Since no HPTLC method is reported for simultaneous estimation of Amlodipine and Atorvastatin calcium in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously. The proposed method was successfully applied for simultaneous determination of atorvastatin and amlodipine in combined dosage forms that are available in market.

MATERIALS AND METHODS

Reagents and chemicals

Atorvastatin calcium was obtained as gift sample from Micro labs and Amlodipine was obtained as gift sample from Cipla Pharmaceuticals. Purified water was prepared using a Millipore Milli-Q (Nanopure Diamond, Barnstead thermolyne, USA) water purification system. Acetonitrile, Methanol was purchased from Merck Ltd. (Mumbai, India)

Instrumentation

CAMAG HPTLC instrument was used in this method. CAMAG HPTLC is equipped with CAMAG TLC scanner-3, Linnomate V Automatic sample applicator controlled by WIN CATS software (1.4.3 version). Aluminum packed silica Gel 60 F_{254} HPTLC plates (20 X 10cm, layer thickness 0.2mm, E.MERCK).

Table 1. Optimized chromatographic conditions

Stationary phase precoated TLC plates	:	Silica gel 60GF_{254}
Mobile phase	:	ethyl acetate: methanol: ammonia
"Mobile phase ratio (%v/v/v)	:	7.5:2:0.5
Saturation time	:	20 minutes.
Solvent front	:	85 mm.
Band length	:	6 mm.
Slit dimension	:	5.00 x 0.45 mm.
Source of radiation	:	Deuterium.
Scan wavelength	:	365 nm.
R_f values		
Atorvastatin calcium	:	0.26 ± 0.02.
Amlodipine besylate	:	0.50 ± 0.02.

Selection of detection wavelength

After chromatographic development, bands were scanned over the range of 200-400 nm and the overlain spectra were obtained. UV spectra of atorvastatin calcium and amlodipine besylate on precoated plate were recorded. The λ_{max} of atorvastatin calcium and amlodipine besylate was found to be 282 nm and 365 nm respectively.

The chromatogram scanned at 365 nm showed higher peak area and better peak shape for both atorvastatin calcium and amlodipine besylate than other wavelengths. So 365 nm was selected as the detection wavelength (Figure 3).

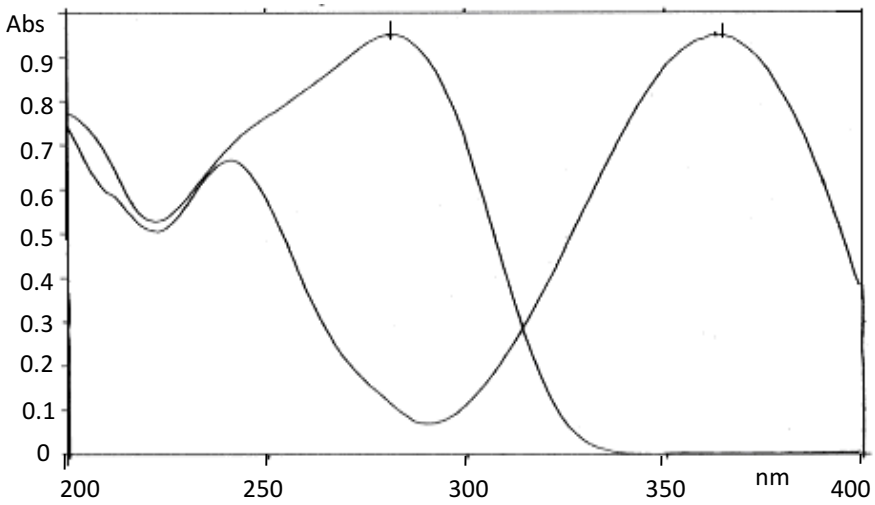

Figure 3: UV spectra of standard atorvastatin calcium and standard amlodipine besylate on TLC plate

Preparation of standard stock solution

Standard stock solution of 10mg and 5mg of atorvastatin calcium and amlodipine besylate mixture was weighed and the average weight was calculated. The drugs were dissolved in methanol and the volume was made up to obtain a final concentration range of 100-500 ng/spot for amlodipine besylate and 200-600 ng/spot for atorvastatin calcium (Figure 4).

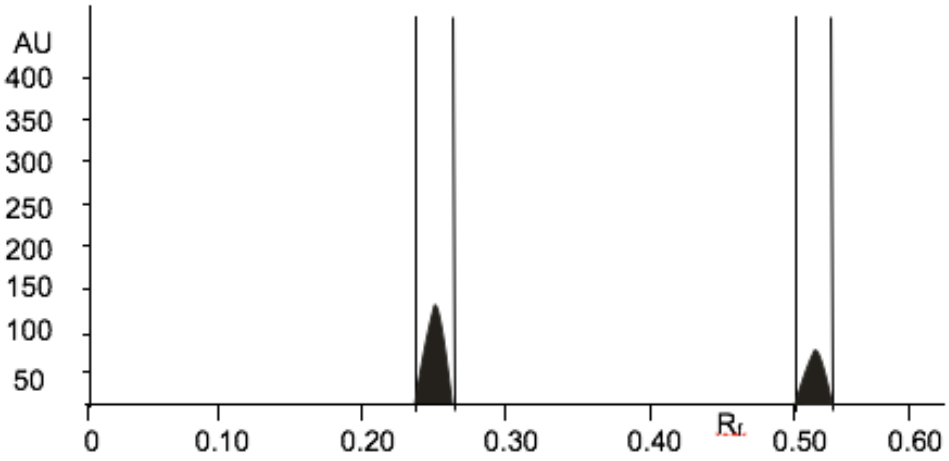

Figure 4. Standard chromatogram of atorvastatin calcium (400ng/spot) and amlodipine besylate (300ng/spot)

Recording of the chromatogram

With the fixed chromatographic conditions standard solutions were applied on the plate, dried, developed analyzed photo metrically and chromatograms recorded. The R_f values of amlodipine besylate and atorvastatin calcium were found to be 0.50 ± 0.02 and 0.26± 0.02 respectively. This was followed by the application of sample solution obtained from the formulation.

Analysis of Tablet Formulation

Twenty tablets each containing quantity equivalent to 10 mg of atorvastatin calcium and 5 mg of amlodipine besylate were weighed and an average weight was taken. The drugs were extracted by the addition of methanol and finally made up to 100ml in a standard flask. The solution was filtered through Whatmann filter paper. After filtering aliquots were spotted on the plate and developed chromatograms were scanned. The peak areas were noted and concentration of sample solution was calculated using respective standard calibration curve (Table 2), (Figure 5).

Table 2. Analysis of formulation

Drug	Amount (mg/tablet)		% Label claimed	% RSD*
	Label claimed	Found		
Atorvastatin calcium	10	9.82	99.2	0.152
Amlodipine besylate	5	4.96	99.1	0.124

**Mean RSD of six observations*

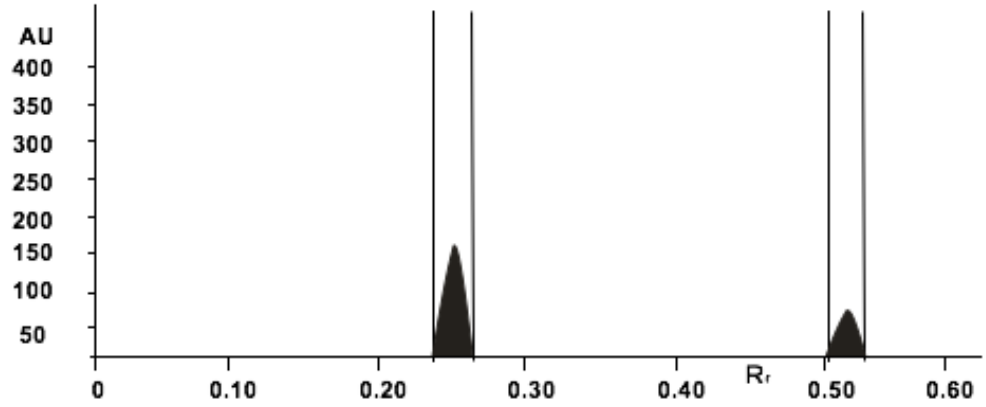

Figure 5. Chromatogram of atorvastatin calcium (400ng/spot) and amlodipine besylate (300ng/spot) in formulation

Validation of the Method

The method was validated by establishing linearity, accuracy, inter day and intra day precision of measurement and repeatability of sample application, robustness and ruggedness. The limit of detection and limit of quantification were also determined [40-44].

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was studied by analyzing five concentrations of the

drug, and process was repeated for five times each. It was done over the concentration range of 100-500 ng/spot for amlodipine besylate and 200-600ng/spot for atorvastatin calcium. The calibration curves were constructed by plotting peak areas versus concentrations with the help of win-CATS software which are shown in graph I and graph II.

Precision

Intraday precision

Intraday precision was found by carrying out the analysis of standard drugs at three different concentrations in the linearity range of the drugs for three times on the same day. Each concentration was applied in triplicates and % RSD was calculated.

Inter day precision

Inter day precision was found by carrying out the analysis of the standard drugs at three different concentrations in the linearity range of the drugs for three days and % RSD was calculated.

Repeatability

Repeatability of sample application

Repeatability of sample application was assumed by spotting 400 ng/ml of drug solution, 6 times on TLC plate followed by development of plate and recording the peak area for 6 spots and % RSD was calculated.

Repeatability of measurement

The repeatability of measurement of peak area was determined by spotting standard drug solution on TLC plate and developing the plate. The spot was scanned 6 times without changing the position of the peak and % RSD was calculated.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at two different levels of 50 % and 100 %. The % recovery and % RSD were calculated.

Limit of Detection and Limit of Quantification (LOD &LOQ)

The sensitivity of measurements of atorvastatin and amlodipine by the use of the proposed method was estimated in terms of the Limit of Quantitation (LOQ) and Limit of Detection (LOD). These were calculated by the use equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration plot.

Standard and sample solution stability

Stability studies were also carried out by keeping the standard and sample solution prepared at

room temperature for several hours and was spotted every time on a fresh plate. After development and scanning the plates were observed for change in peak areas and appearance of additional peaks. The RSD was calculated.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on TLC scanner 3 in the range of 200-400 nm using WinCats software (version 1.4.3).

Method Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was determined by small deliberate changes in mobile phase composition (± 2%), chamber saturation period (± 10%), and development distance (± 10%). The time from spotting to chromatography and from chromatography to scanning was varied from 10 min. When very small changes were made to the method conditions there were no marked changes in chromatographic behavior and content of the drug, % RSD was calculated.

Method Ruggedness

Ruggedness test was determined between two different analysts and instruments. The value of Percentage RSD was calculated.

RESULTS AND DISCUSSION

Method development

It was observed that both drugs showed considerable absorbance at 365 nm. So, 365 nm was selected as the wavelength for detection. Method development for resolution of atorvastatin calcium and amlodipine was started with the development of densitogram with neat solvents in different ratios and combinations of Propanol, Ethyl acetate, Methanol, n-butanol, Triethylamine, and Glacial acetic acid. Finally, ethyl acetate: methanol: ammonia (7.5:2:0.5 v/v/v) was selected as a mobile phase with a good resolution at Rf 0.26 ± 0.02 and 0.50 ± 0.02 for atorvastatin calcium and amlodipine respectively.

Validation

Linearity and Range

The linear regression data showed a good linear relationship over a concentration range of 100-500 ng/spot for amlodipine besylate **(Figure 6)** and 200-600 ng/spot for atorvastatin calcium **(Figure 7)**. The slope, intercept and correlation coefficient values of amlodipine besylate were found to be 2.30, 80.68, and 0.9983 respectively and 1.4715, 0.7008 and 0.9994 respectively for atorvastatin calcium. The results are shown in Table 3.

Table 3: Regression analysis of the calibration curves for amlodipine and atorvastatin calcium for the proposed HPTLC method

Parameters	Amlodipine	Atorvastatin calcium
Linear Range(ng/spot)	100-500ng/spot	200-600ng/spot
Slope	2.30	1.4715
Intercept	80.68	0.7008
Regression co-efficient (r^2)	0.9983	0.9994

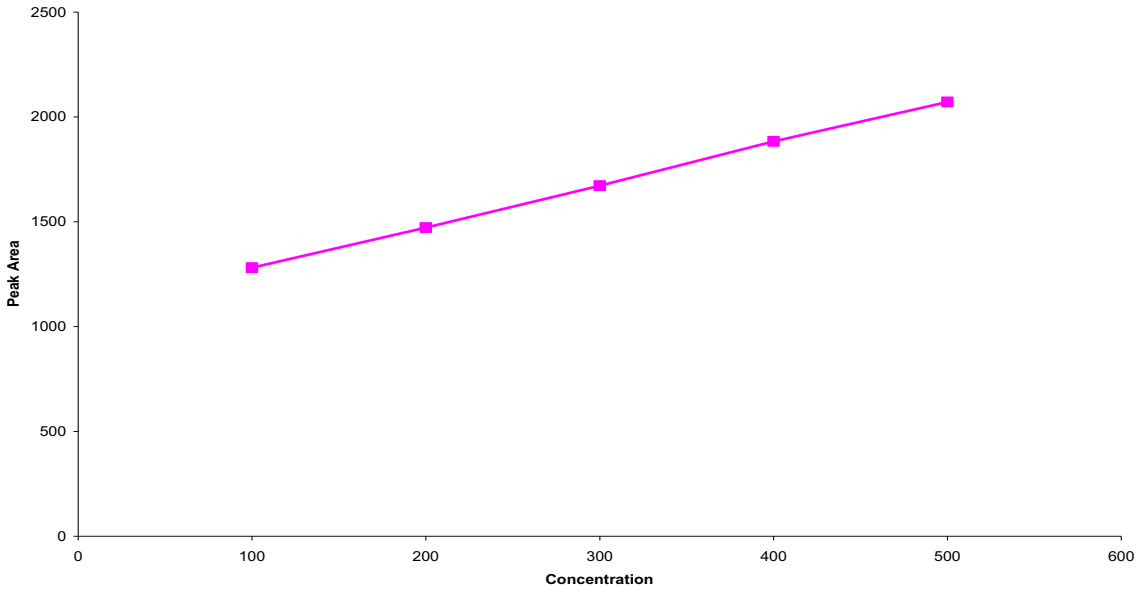

Figure 6. Calibration curve of amlodipine besylate

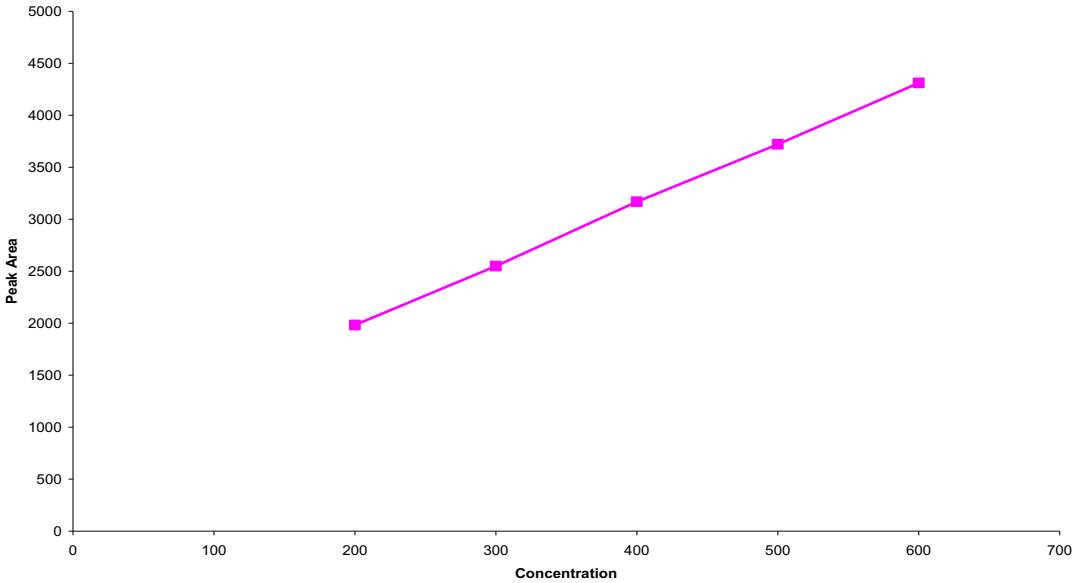

Figure 7. Calibration curve of atorvastatin calcium

Precision

Precision was calculated as interday and intraday variations. The RSD (Relative Standard Deviation) was found to be not more than 1 % for both intraday and Interday precision (Table 4 & 5).

Table 4. Intra day precision

Volume applied (ng/ spot)	Amlodipine besylate		Atorvastatin calcium	
	Peak area	% RSD	Peak area	% RSD*
200	1483.3	0.5071	1983.4	0.5175
	1485.3		1968.8	
	1472.3		1992.2	
300	1683.1	0.6254	2549.3	0.5610
	1665.8		2560.2	
	1660.5		2589.7	
400	1821.6	0.5147	3168.8	0.6037
	1809.8		3117.6	
	1811.2		3145.1	

**Mean RSD of three observations*

Table 5. Inter day precision

Volume applied (ng/spot)	Day	Peak Area		% RSD*	
		Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium
200	1	1472.3	1961.3	0.7267	0.5175
	2	1487.2	1945.6		
	3	1468.2	1970.8		
300	1	1636.9	2538.3	0.8666	0.5610
	2	1599.3	2548.2		
	3	1635.2	2575.7		
400	1	1883.3	3168.8	0.7140	0.6037
	2	1872.6	3117.6		
	3	1885.8	3145.1		

**Mean RSD of three observations*

Repeatability

The repeatability showed excellent % RSD less than 0.60 after six applications **(Table 6 & 7).**

Table 6. Repeatability of sample application

Volume applied (ng/spot)	Peak Area		% RSD*	
	Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium
400	1868.3	3145.3	0.4493	0.5493
	1855.5	3175,3		
	1827.6	3131.6		
	1835.1	3137.9		
	1815.6	3151.8		
	1841.7	3137.9		

**Mean RSD of six observations*

Accuracy

The percentage recovery for amlodipine besylate was found to be 101.82 (at 50%), 99.12 (at 100%) with % RSD values ranging from 0.112 to 0.117 and 101.41

(at 50%), 101.71 (at 100%) for atorvastatin calcium with % RSD values ranging from 0.100 to 0.124 **(Table 8)**.

Table 7. Repeatability of measure

Concentration (ng/spot)	Peak Area		% RSD*	
	Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium
400	1885.6 1881.3 1881.2 1871.3 1875.5 1874.2	3152.8 3137.6 3174.4 3133.6 3151.8 3150.3	0.2562	0.3340

**Mean RSD of six observations*

Table 8. Accuracy

Levels	% of Recovery		% RSD*	
	Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium
50%	101.82	101.41	0.112	0.100
100%	99.12	101.71	0.117	0.124
150 %	100.5	99.5	0.251	0.249

**Mean RSD of six observations*

LOD & LOQ

'Limit of Detection' was found to be 30ng/spot and 60ng/spot for amlodipine besylate and atorvastatin calcium respectively Figure 8 & 9). Where as 'Limit of Quantification' was found to be 100ng/spot and 200ng/spot for amlodipine besylate and atorvastatin calcium respectively (Figure 10-11).

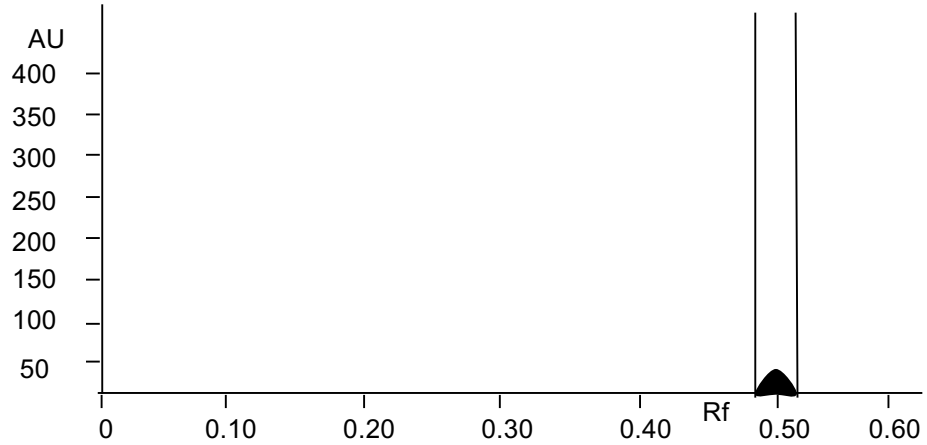

Figure 8. Limit of Detection of amlodipine besylate

Standard and sample solution stability

Analyte should not decompose during development of the chromatogram and should be stable in solution as well as the solvent. The RSD was found below 2%. It was observed that the plates were stable up to 2 hours (Table 9).

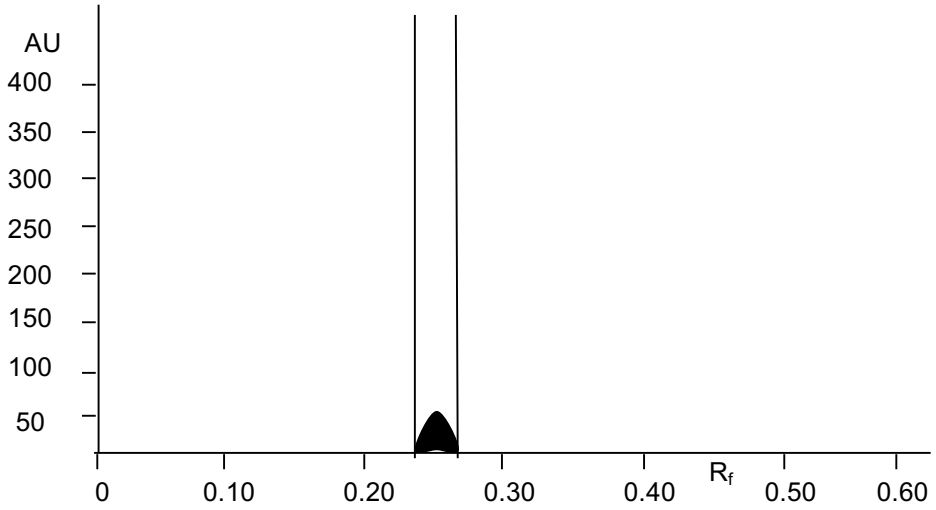

Figure 9. Limit of Detection of atorvastatin ca

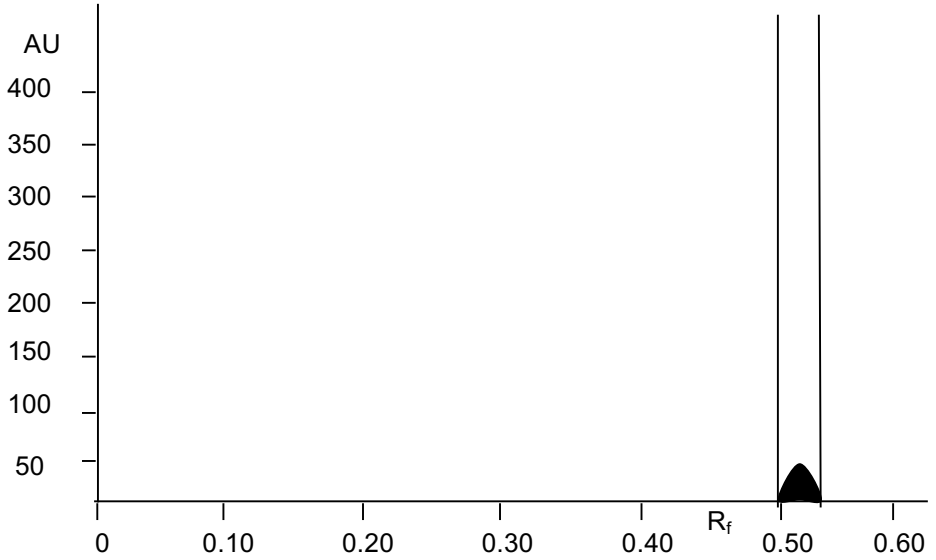

Figure10. Limit of Quantification of amlodipine besylate

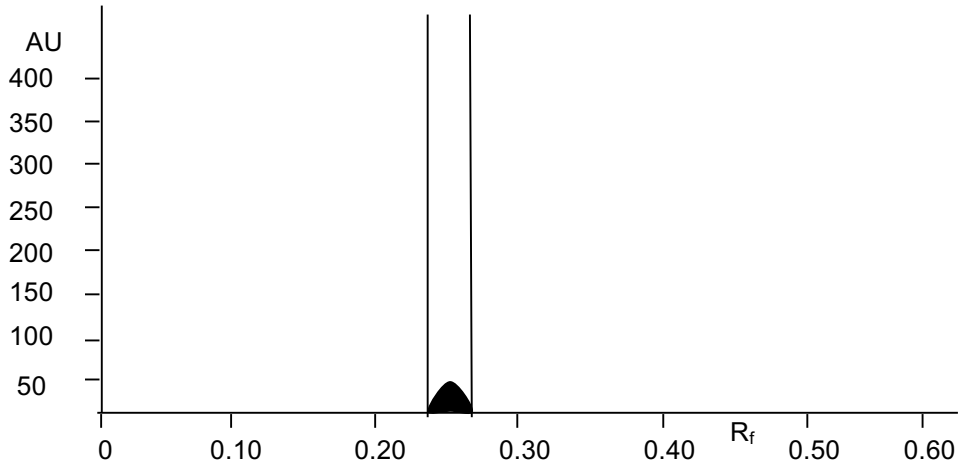

Figure 11. Limit of Quantification of atorvastatin calcium

Table 9. Stability of the plate

Volume applied (ng/spot)	Time in (hrs)	Peak area	
		Amlodipine besylate	Atorvastatin calcium
400	0	1885.6	3137.6
	½	1883.7	3142.7
	1	1872.3	3142.8
	1 ½	1886.9	3147.9
	2	1882.5	3121.5

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s, m) = 0.9983 and r (m, e) = 0.9994, indicating the non interference of any other peak of degradation product, impurity or matrix. Peak purity was found to be more than 0.995, which demonstrated that the method is specific.

Robustness

There were no significant changes in Rf and peak areas, which demonstrated that the developed HPTLC method is robust.

Ruggedness

The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method.

CONCLUSION

Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of amlodipine and atorvastatin calcium as bulk drug and in pharmaceutical formulation without any interference from the excipients. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control setup providing all the parameters are followed accurately for its intended use.

ACKNOWLEDGEMENT

The authors thank for Micro Labs and Cipla Pharmaceuticals providing the gift samples of atorvastatin and amlodipine respectively.

REFERENCES

1. Budawari S. (2008). *The Merck Index*, 23rd ed. Whitehouse Station, New Jersey. 516, 6235.
2. Martindale & Sweetman SC. (1999). *The complete drug reference*. 32nd ed. Pharmaceutical Press. 822, 907.
3. Oneil M.J, Smith A, Heckelman P.E & Budawari S. (2001). *The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals*, 13rd ed., Merck and Co Inc., White House Station, New jersey. 488, 865.

4. Mohammadi A., Rezanour N., Ansari M., Dogaheh, Ghorbani F., Bidkorbeh., Hashem M.& Walker RB. (2007). Stability indicating RP-HPLC assay for simultaneous determination of Atorvastatin and Amlodipine in commercial tablets. *Journal of Chromatography B*. 846, 215-221.
5. Desager J.P. & Hormans Y. (1996). Clinical Pharmacokinetics of 3- hydroxy-3methylglutaryl-coenzyme A reductase inhibitors. *Clin. Pharmacokinet*. 31, 348-371.
6. Malinowski J.M. (1998). Atorvastatin, A hydroxymethylglutarylcoenzyme A reductase inhibitors. *J.Health-Syst Pharm*. 2253-2267.
7. Lipitor product information. Park-Davis/Pfizer Inc. April (2002).
8. Burnham T.H. (2002). HMG-CoA reductase inhibitors. In. ed. Drug Facts and Comparisons. *Louis Facts and Comparisons*, Inc. 536-542a.
9. *Indian Pharmacopoeia*, (2007). Govt. of India, Ministry of Health and Family Welfare, Vol. 2, Delhi. Publication by Controller of Publication. 749-52.
10. *Indian Pharmcopoeia*, (2007). Govt. of India, Ministry of Health and Family Welfare, Vol. 2, Delhi. Publication by Controller of Publication.714-16.
11. *British Pharmacopoeia*, (2005). International ed. Published on the Recommendation of the Medicines Comissions Pursuant to Medicines Act vol. 1, 138.
12. *The European Pharmacopoeia*, (2002). Counsile of Europe, Codex,France, 4th Edn.639-40.
13. *The United States Pharmacopoeia Convention*, Inc. (2007). Rockville, M.D. 3496-97, 1532.
14. Zarghi A., Shafaati A., Foroutan S.M. & Khoddam A., (2005). A simple and rapid HPLC method for the determination of atorvastatin in human plasma with UV detection and its application to pharmacokinetic studies, *Arznei mittel forschung*. 55, 451-454.
15. Koytchev R., Ozalp Y., Erenmemisoglu A.,Vander Meer M.J.& Alpan R.S.(2004). Bioequivalence study of atorvastatin tablets, *Arzneimittelforschung.* 54, 573-577.
16. Bahrami G., Mohammadi B., Mirzaeei S. & Kiani A. (2005). Determination of atorvastatin in human serum by reversed-phase high-performance liquid chromatography with UV detection, *J. Chromatogra. B*. 826, 41-45.
17. Hermann M., Christensen H. & Reubsaet J. L. (2005). Determination of atorvastatin and metabolites in human plasma with solid-phase extraction followed by LC-tandem MS, *Anal. Bioanal. Chem.* 382, 1242-1249.
18. Jemal M., Ouyang Z., Chen B. C., & Teitz D. (1999). Quantitation of the acid and lactone forms of atorvastatin and its biotransformation products in human serum by high-performance liquid chromatography with electrospray tandem mass spectrometry, *Rapid Commun. Mass Spectrom*. 13, 1003-1015.
19. Bullen W. W. & Miller R. A. (1999). Development and validation of a high-performance liquid chromatography tandem mass spectrometry assay for atorvastatin, ortho-hydroxy atorvastatin, and para-hydroxy atorvastatin in human, dog, and rat plasma, *J. Am. Soc. Mass Spectrom.* 10, 55-66.
20. Erturk S., Sevinc A. E., Ersoy L. & Ficicioglu S. (2003). An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets, *J. Pharm. Biomed. Anal.* 33, 1017-1023.
21. Pasha M. K., Muzeeb S., Basha S. J., Shashikumar D., Mullangi R. & Srinivas N. R. (2006). Analysis of five HMG-CoA reductase inhibitors- atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and in vitro metabolism studies, *Biomed. Chromatogr*. 20, 282-293.
22. Manoj K., Shanmugapandiyan P. & Anbazhagan S. (2000). RP-HPLC method for simultaneous estimation of atorvastatin and aspirin from capsule formulation, *Indian Drugs*. 41, 284-288
23. Khopde S.A. & Jain N.K. (2000). Difference spectrophotometric estimation of amlodipine besylate, *Indian Drugs*. 37, 351-53.
24. Bahrami G. & Mirzaeei S.(2004). Simple and rapid HPLC method for determination of amlodipine in human serum with fluorescence detection and its use in pharmacokinetic studies, *J. Pharm. Biomed. Anal*. 36, 163-68.
25. Streel B., Laine C., Zimmer C., Sibenaler R. & Ceccato A. (2002). Enantiomeric determination of amlodipine in human plasma by liquid chromatography coupled to tandem mass spectrometry, *J. Biochem. Biophys. Methods*. 54, 357-68.

26. Carvalho M., Oliveira C. H., Mendes G. D., Sucupira M., Moraes M. E. & De Nucci G. (2001). Amlodipine bioequivalence study: quantification by liquid chromatography coupled to tandem mass spectrometry, *Biopharm. Drug. Dispos*. 22, 383-90.
27. Patki R. V., Tamhankar C. P. & Tipnis H. P. (1994). Simple and rapid high performance liquid chromatographic estimation of amlodipine from pharmaceutical dosage, *Indian Drugs*. 31,560-61.
28. Avadhanulu A. B., Srinivas J. S. & Anjaneyulu Y. (1996). RP-HPLC determination of amlodipine besylate in drug and its pharmaceutical dosage forms, *Indian Drugs*. 33, 36-40.
29. Zarapkar S. S., Katle S. S. & Rane S. H. (1997). HPLC determination of amlodipine and atenolol simultaneously from pharmaceutical preparation, *Indian Drugs*. 34, 350-53.
30. Valiyare G. R., Chandra A., Apte S. K. & Mahadik A. A. (2005). HPLC determination of amlodipine, losartan and ramipril in pharmaceutical formulations, *Indian Drugs*. 42, 309-312.
31. Kamble N. & Venkatachalam A. (2004). Determination and validation of HPLC method for simultaneous determination of lisinopril and amlodipine from tablet, *Indian Drugs*. 41, 179-781.
32. Kulkarni A. P., Gat G. V., Pimple S.V. & Joshi M.A. (2003). HPLC method for determination of losartan potassium and amlodipine besylate in tablets, *Indian Drugs*. 40, 298-299.
33. Zarapkar S.S. & Kanyawar N. S. (2002). Simultaneous estimation of amlodipine besylate and losartan potassium in pharmaceutical dosage by RP-HPLC, *Indian Drugs*. 39, 338-41.
34. Altiokka G. & Altiokka M. (2002). Flow injection analysis of amlodipine using UV-detection, *Pharmazie*. 57, 500-503.
35. Kamat K. & Chaturvedi S. C. (2005). Stability indicating assay method for amlodipine tablets, *Indian J.Pharm. Sci*. 67, 236-239.
36. Naidu K. K., Kale U. N. & Shingare M. S. (2005). Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product. *J. Pharm. Biomed. Anal.* 39, 147- 155.
37. Sahu R. & Patel V. B. (2006). Simultaneous spectrophotometric determination of amlodipine besylate and atorvastatin calcium from their binary mixture by dual wavelength and zero absorbance measurement, *Indian Drugs*. 43, 160-161.
38. Chaudhari B. G. & Patel N. M. (2006). Development and Validation of HPLC method for simultaneous estimation of Atorvastatin Calcium and Amlodipine Besylate. *J. Pharm. Research*. 5, 141-144.
39. Chaudhari B. G., Patel N. M. & Shah P. B. (2007). Stability Indicating RP-HPLC for simultaneous determination of atorvastatin calcium and amlodipine besylate from their combination drug Products, *Chem. Pharm. Bull*. 55, 241-246.
40. ICH Q2A (1994). Text on Validation of analytical procedures, *International Conference on Harmonization tripartite guidelines*. adapted 27 Oct .
41. Sethi P. D. (1996). *High performance thin layer chromatography Quantitative Analysis Pharmaceutical formulations,* 1st Edition, CBS Publishers and distributors, India.
42. Touchstone J .C. (1992). *Practice of thin layer chromatography,* 3rd Ed., Published by John Wiley and sons, Inc., USA.
43. Sethi P. D & Charegaonkar D. (1999). *Identification of drugs in pharmaceutical formulations by thin layer chromatography,* 2nd Ed., CBS Publishers and distributors, USA.
44. ICH Q2B Text on validation of analytical procedures. (1994). Methodology *International Conference on Harmonization*. Harmonization tripartite guidelines, adapted 27 Oct.

◈◈◈