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DEVELOPMENT AND VALIDATION OF RP-HPLC AND SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND AMLODIPINE IN PHARMACEUTICAL DOSAGE FORMS

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Amlodipine (AML) and Diclofenac,
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C_{18} (4.6*250) mm,
5 micron column,
Validation

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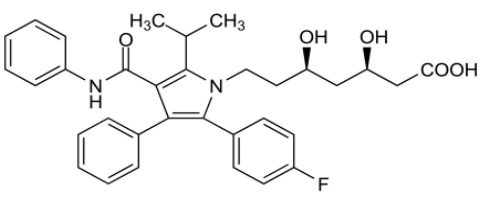
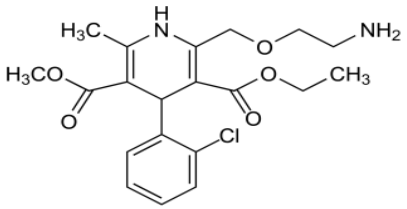
ABSTRACT

A simple, rapid, precise, accurate, sensitive and time consuming reverse phase high performance liquid chromatography (RP-HPLC) and spectrophotometric method for simultaneous estimation of atorvastatin and amlodipine in pharmaceutical dosage forms have been developed and validated. Drug was resolved on a C_{18} column (Phenomenex phenyl hexyl column, 250mm* 4.6mm i.d, 5um). Utilizing mobile phase of water with 0.4%v/v triethyl amine and acetonitrile with diluted orthophosphoric acid pH adjusted to 5.2 in a ratio of 52.5:47.5 of water and acetonitrile respectively. Mobile phase was delivered at the flow rate of 1.0ml/min. Ultraviolet detection was carried out at 229nm. Separation was completed within 7.75 minutes. Calibration curve was linear with correlation coefficient (r^2) =0.999. Over a concentration range 5-25ug/ml, using diclofenac 10ug/ml as IS. Recovery was between 99.26, 100.2 percentage. In spectrophotometric method it involves the formation and solvation of simultaneous equation at 242nm for Atorvastatin and 256nmfor Amlodipine using Acetonitrile and Water (10:90) as mobile phase. The standard deviation was found to be less than 1% for the assay of tablet. The proposed methods were successfully employed for the estimation of Atorvastatin and Amlodipine in combined tablet formulation.

INTRODUCTION: Atorvastatin (fig-I) is chemically [R-(R*, R*0] [R-(R*, R*0] -2-(4-Fluorophenyl)- β, γ-dehydroxy- 5- (1-mehtylethyl)- 3- phenyl-4- [9phenyl amino)-carbonyl]-1H-pyrrole-1-heptanoic acid. Lipid lowering agent. Amlodipine (**Fig. 1**) is chemically 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester act as a calcium channel blocker [1-4].



ATORVASTATIN



AMLODIPINE

FIGURE 1: STRUCTURE OF ATORVASTATIN AND AMLODIPINE

MATERIALS AND REAGENTS: Atorvastatin and Amlodipine standards were obtained from Zydus Medica Laboratories, Ltd. (Ahmedabad, India), methanol, acetonitrile and buffer (HPLC grade) were obtained from Qualigens Fine Chemicals (Mumbai, India). The (A^2) tablets (Zydus Medica laboratories) of

the combination of atorvastatin and amlodipine were purchased commercially. Double distilled water was used throughout the experiment. Other chemicals used were have analytical or HPLC grade.

Chromatographic Conditions: A chromatographic system prominence consisting of quaternary solvent delivery pump, a degasser and column oven and photodiode array detector, LC20-AT series, C_{18} (4.6*250) mm, 5 micron column was used. The instrumental settings were a flow of 1Ml/min. The injection volume was 10ul. The detection wavelength was 229 nm for all three analytes.The peak purity was checked with the photodiode array detector from LC20-AT.

Mobile Phase: The mobile phase consisted of buffer and acetonitrile in the ratio of 52.5:47.5(v/v). The pH of the mobile phase was adjusted to 5.2mM (v/v) of ortho phosphoric acid in the double distilled water. The mobile phase was mixed and filtered through a nylon filter and degassed.

Standard stock solution: Standard stock solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentrations. Diluents used for the standards and sample preparations were prepared as follows. Diluents were composed of water and acetonitrile in the ratio of 50:50 (v/v) and diluents b was composed of buffer and acetonitrile in the ratio of 52.5:47.5 (v/v) respectively.

Preparation of Standard Solution: 10 mg of Amlodipine was taken in a 10 ml standard flask. To this 2 ml of mobile phase was added for dissolving the drug. Shake it for one min, to get a clear solution and make up the volume to 10 ml with mobile phase (stock solution A).

10 mg of Atorvastatin was taken in a 10 ml standard flask and diluted with few ml of mobile phase until the sample dissolves completely and make up the volume to 10 ml with mobile phase (stock solution B).

The internal standard solution was prepared by taking 10 mg of diclofenac in a 10 ml standard flask. It is dissolved by adding 3 ml of mobile phase, shake it for few minutes to get a clear solution and make up the final volume to 10 ml with mobile phase.

The final standard solution was prepared in such a way that each standard flask contains 5, 10, 15, 20 and 25 µg of amlodipine and atorvastatin and 10µg of diclofenac (IS).

Preparation of Formulation Solutions: Twenty tablets containing 5 mg of each amlodipine and atorvastatin were weighed and finely powdered. From the powdered tablets, a quantity of powdered equivalent to 10mg was weighed. Then, was extracted with 25ml of mobile phase the solution was then filtered and diluted to 1000µg/ml with mobile phase. From this final dilution were prepared which contain each 10µg/ml of both drugs and internal standard.

Procedure: (Spectrophotometric)

Preparation of Standard Stock Solution: Standard stock solution of Atorvastatin and Amlodipine was prepared by weighing each 10mg of atorvastatin and Amlodipine were, transferred to a 10ml volumetric flask and volume made to 10ml with to get a concentration of 1mg/ml, with acetonitrile and water in the ratio of (10:90) from this solution, an aliquot of 1ml was withdrawn, and it was further diluted to 10ml with water (100ug/ml).

Preparation of Standard Solution: For the preparation of calibration curves, stock solution of Atorvastatin and Amlodipine in the concentration range of 7.5-37.5µg/ml was taken respectively for Atorvastatin and Amlodipine. For both drugs 0.75ml, 1.5ml, 2.25ml, 3.ml and 3.75ml were withdrawn from standard stock solution contain (100µg/ml) and make to 10ml with water so that concentration of these solution contain 7.5µg/ml, 15µg/ml, 22.5µg/ml, 30µg/ml, 37.5µg/ml.

Preparation of Sample Solution: Twenty Tablets of brand A^2 (Zydus Medica), label claim 5mg of Atorvastatin, and 5mg of Amlodipine were weighed, average weight determined and finely powdered. Appropriate quantity of powder from each tablet equivalent to 5mg were weighed and finely powdered. The mixture was then extracted wit acetonitrile and water, then extract was filtered through Whatmann filter no. 41 and filtrate was appropriately diluted to get final concentration 7.5µg/ml to 37.5µg/ml. Absorbance of this solution was measured at 242nm and 256nm.

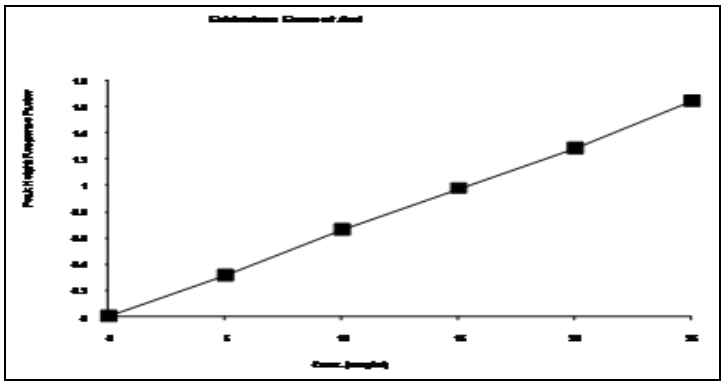
Method: Two wavelength selected to frame the simultaneous equation method were at 242nm and 256nm since these two wavelength ratio of the absorptivity of two component or maximum. For calibration curves, stock solutions of Atorvastatin and Amlodipine in the concentration of range of 7.5 – 37.5µg/ml respectively. The absorbance of this atorvastatin and amlodipine was measured at 242nm, and 256nm calibration curve were plotted (**Fig. 2, 3**). The absorptive of both the drugs at both the wave length were determined.

The absorbance and the absorptivity values at the particular wavelength were calculated and substituted in the following equation, to obtain the concentration.

$C_{ATR} = (A_1ax_2 – A_2ax_1) / (ax_2ay_1 – ax_1ay_2)$.

$C_{AML} = (A_2ay_1 – A_1ay_2) / (ax_2ay_1 – ax_1ay_2)$……..1

Where, C_{ATR}, C_{AML} are concentration of Atorvastatin and Amlodipine respectively, A_1 is the absorbance of sample at 242nm, A_2 is the absorbance of sample at 256nm, ax_1 is the absorptivity of Atorvastatin at 242nm and ax_2 is the absorptivity of Atorvastatin at 256nm, ay_1 is the absorptivity of Amlodipine at 256nm and ay_2 is the absorptivity of Amlodipine at 242nm.



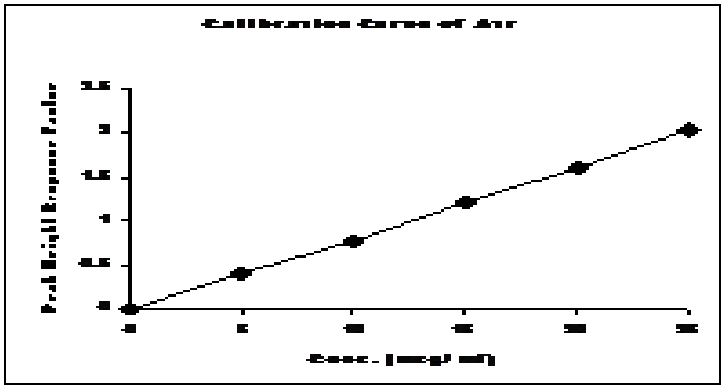


FIG. 2 & 3: CALIBRATION CURVE OF ATORVASTATIN AND AMLODIPINE

Results and Discussion of HPLC and Spectrophotometric Method:

HPLC Method:

Validation of the Method: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and presented in **Table 1**. The chromatogram of the recovery studies was recorded. From the data obtained, recoveries of standard drugs were found to be accurate and are within the specified limits.

TABLE 1: ACCURACY (RECOVERY STUDIES)

Drug	% Recovery*		% RSD*	
	50% level	100% level	50% level	100% level
Atorvastatin	99.26	100.1	0.60	0.10
Amlodipine	99.93	100.2	0.04	0.04

*Mean of three replicates

The precision of the method was determined by studying repeatability and reproducibility. The response factor of drug peaks and percentage relative standard deviation were calculated and presented in **Table 3 & 4**. The results revealed that the method developed is reproducible.

The standard drug solutions in varying concentrations ranging from 50 to 150% of the targeted level of the assay concentration containing internal standard were examined by the assay procedure. The linearity and range for both the drugs was found to be from 5 to 25 µg/ml.

Estimation: Estimation of Atorvastatin and Amlodipine in dosage forms by High Performance Liquid Chromatography was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared and chromatograms were recorded. The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean, and standard deviation in formulations were calculated and presented in **Table 2**. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

TABLE 2: ANALYSIS OF FORMULATION

Drug	Label Claim (mg/tablet)	Estimated Amount (mg/tablet)*	% Label claim*	%RSD*
Atorvastatin	5	4.96	99.6	0.32
Amlodipine	5	4.90	98.4	0.30

*Mean of three replicates

Determination of Active Ingredients in Tablets: The contents of two drugs in tablets were determined by the proposed method using the calibration curve. The results are shown in **Table 5**. The chromatogram of the tablet sample is shown in (**Figure 4**).

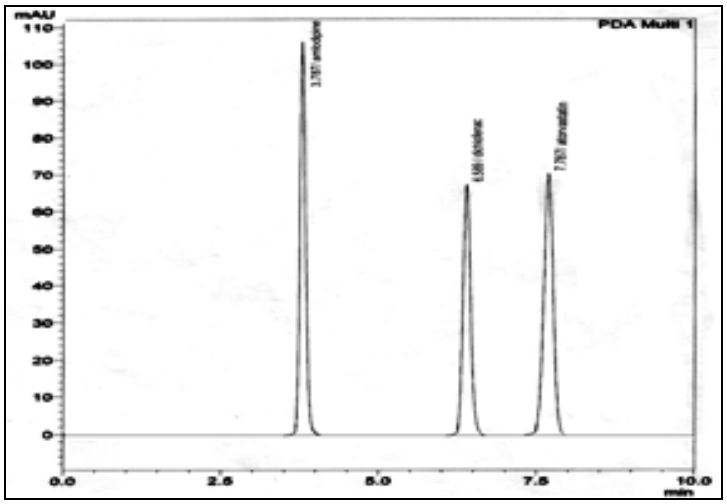


FIGURE 4: A TYPICAL CHROMATOGRAM OF THE TABLET: AMLODIPINE (3.7), DICLOFENAC (6.5), ATORVASTATIN (7.7)

Repeatability of injection: A standard solution of mixture of drugs was injected 6 times and its % RSD was calculated, **table 4**.

The response factor, slope, intercept and correlation coefficient values were calculated. The correlation coefficient of Atorvastatin and Amlodipine were found to be 0.9990 and 0.9997 respectively. The calibration curves were plotted using response factor Vs. concentration of the standard solutions (**Fig. 4 and 5**). The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use.

The LOD and LOQ of the developed method were determined by analysing progressively low concentration of the standard solutions using the developed methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). LOD of Atorvastatin and Amlodipine were found to be 450 and 475 ng/ml. the LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Atorvastatin and Amlodipine were found to be 1300 and 1425 ng/ml. The resolution, capacity factor, theoretical plates/meter, peak symmetry was calculated for the standard solutions and is presented in Table 5. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

TABLE 3: LINEARITY AND RANGE IN HPLC

Internal Standard Peak area (10μg/ml)	Atorvastatin			Amlodipine		
	Concentration (μg/ml)	Peak area	Response factor	Concentration (μg/ml)	Peak area	Response factor
599358	5	135930	0.406	5	185201	0.309
628024	10	399470	0.782	10	272935	0.659
598449	15	762008	1.218	15	508188	0.977
567634	20	1283839	1.701	20	830062	1.286
584444	25	2154909	2.030	25	1596526	1.645

TABLE 4: REPEATABILITY OF INJECTION

Concentration (μg/ml)	Injection	Peak area		% RSD*	
		ATR	AML	ATR	AML
Atorvastatin (10 μg/ml); Amlodipine (10 μg/ml)	1	510576	414700	0.3	0.08
	2	514282	414830		
	3	514640	413900		
	4	514150	414682		
	5	514219	414462		
	6	514119	414500		

*Mean of three replicates

TABLE 5 : SYSTEM SUITABILITY STUDIES

Drug	Rs	N	K'	Tailing factor	HETP	LODng/ml	LOQng/ml
Atorvastatin	4.793	10537	1.055	1.045	14.235	450	1300
Amlodipine	_	6390	_	1.223	23.473	475	1425

Rs = Resolution, N = Theoretical plate, K'= Capacity factor

Spectrophotometric Method: (Table 6 & 7)

TABLE: 6 SUMMARIES OF VALIDATION PARAMETERS

PARAMETERS	ATORVASTATIN	AMLODIPINE
Linearity range (µg / ml)	7.5-37.5	7.5-37.5
Correlation coefficient (r)	0.999	0.999
Intercept	0.00020	0.0000
Slope	0.03467	0.0320
Standard deviation (SD)	0.4111	0.3795
Standard error (SE)	0.1838	0.1697
Limit of detection(LOD)	0.025	0.024
Limit of quantification (LOQ)	0.076	0.070
Repeatiblity in %rsd*	8.5	6.2
% Recovery(std mixture)*	97.92 %	98.25%
Interday in %rsd*	1.10±0.05	1.02±0.006
Intraday in% rsd*	0.50±0.05	0.40±0.08

*Mean of three replicates

TABLE: 7 ANALYSIS OF FORMULATION

Formulations	Method	
A^2	ATORVASTATIN	AMLODIPINE
%recovery*	98.51%	97.48%
Standard deviation (SD)	0.4000	0.3305
Standard error (SE)	0.2309	0.1908
Coefficient of variation	0.41	0.34

*Mean of three replicates

SUMMARY AND CONCLUSION:

HPLC: The scope and objective of the present work is to optimize the chromatographic conditions, to develop HPLC& spectrophotometric method for the estimation of drugs in selected multi-component dosage form and the same is validated [5-16].

Various solvent systems were tried among which water and (0.4%v/v Triethylamine): Acetonitrile with ratio 52.5:47.5, pH 5 was selected as mobile phase, which gave good resolution and peak shapes. The flow rate was set at 1.0 ml/min. detection was carried out by PDA detector at 229nm. Quantitation was done by internal calibration method. At the optimum conditions mentioned above, Diclofenac was selected as internal standard for the analysis.

The linearity and range was established over the range of 5 to 25 µg/ml for both Atorvastatin and Amlodipine. The correlation coefficient of Atorvastatin and Amlodipine were found to be 0.999 and 0.999. The method was validated for accuracy, precision, and system suitability. The percentage recovery of Atorvastatin and Amlodipine was found to be 99.26 % and 99.93 % at 50% level and 100.1% and 100.2% at 100% level. The low standard deviation value and good percentage recovery indicates the reproducibility and accuracy of the developed method. Similarly the RSD value for precision was also within the acceptable limit.

The developed HPLC method requires less time, no tedious extraction procedure were involved, run time were less than 10 min, suitable for the analysis of raw material.

Hence, the chromatographic method developed for atorvastatin and amlodipine is said to be simple, precise and accurate that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

Spectrophotometric Method: The proposed methods were successfully used to estimate the amount of Atorvastatin and Amlodipine, present in the marketed tablet formulations The assay values for both the tablets were compared with corresponding labeled amounts and the validation parameters of proposed methods are summarized in Table 6 & 7. The result of analysis has been validated statistically and by recovery studies. The standard deviation was found to be less than 1% for the assay of tablet. The method was found to be simple, accurate, rapid and economical and proposed method can be employed for the routine analysis of tablets.

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