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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS DETERMINATION OF ATORVASTATIN CALCIUM AND AMLODIPINE BESILATE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A high performance liquid chromatography method was developed for the simultaneous separation and quantization of Atorvastatin calcium and Amlodipine besilate in combined pharmaceutical tablet dosage form. The chromatographic estimation was achieved by using mobile phase consisting of a mixture of phosphate buffer (1ml ortho phosphoric acid in 1000 ml 0f water) acetonitrile and methanol (53:43:4, v/v) at a flow rate of 1 ml/min and UV detection at 246 nm, using photodiode array detector. The determination was carried out by using Grace Smart RP C-18 column (250 X 4.6 mm, 5 µm). The retention time of Amlodipine besilate and Atorvastatin calcium was 3.337 and 6.067 respectively. Amlodipine besilate and Atorvastatin calcium calibration curves were linear with correlation coefficient 0.9989 and 0.9981 over a concentration range of 40-60 µg/ml and 80-120 µg/ml respectively. Recovery was between 99.60-100.02 for AML and 99.05-100.52% for ATV, respectively.

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INTRODUCTION: Atorvastatin calcium [1], chemically, [R-(R*, R*)]-2- (4- fluorophenyl) - β, δ- dihydroxy 5 (1- methylethyl)- 3- phenyl- 4- [(phenyl amino) carbonyl]- 1H pyrrole- 1- heptanoic acid, calcium salt trihydrate (2:1) is an antihyperlipoproteinemic drug [2, 3], used for treatment of hypercholesterolemia [**Fig. 1**].

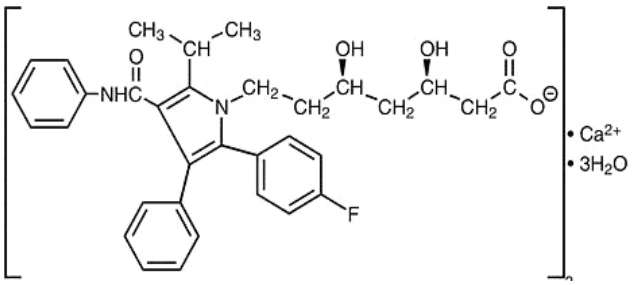

FIG. 1: ATORVASTATIN CALCIUM

Amlodipine [4], chemically, 2- [(2-Aminomethoxy) methyl] - 4- (2- chlorophenyl) - 1, 4- dihydro-6- methyl-3, 5- pyridine dicarboxylic acid, 3- ethyl- 5-methyl ester, is a calcium channel antagonist, used as an anti-hypertensive drug [5] [**Fig. 2**].

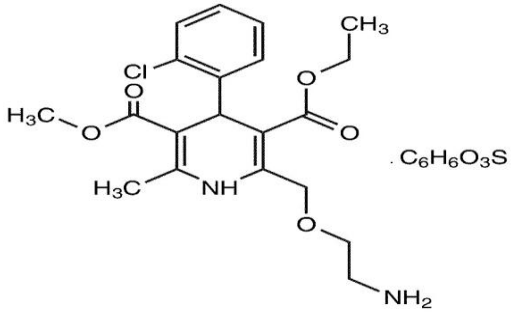

FIG. 2: AMLODIPINE BESYLATE

Literature survey reveals that analytical method, [6, 7] including Capillary zone electrophoresis, [8] and HPLC [9] methods, are available for the determination of Atorvastatin in pharmaceutical dosage forms. The combination of Atorvastatin (ATV) and Amlodipine (AML) has recently been introduced into the market. This combination of Amlodipine and Atorvastatin can be safely used in the treatment of patients with concomitant hypertension and dyslipidemia [10- 15]. However, so far, no method was reported for the simultaneous estimation of Atorvastatin and Amlodipine, in combination [16-18]. The aim of this study was to develop and validate a rapid, simple and specific method by HPLC with UV detection, to quantify the concentration of both Atorvastatin and Amlodipine in table dosage forms. The method validation was performed according to the FDA guidelines for the analytical methods [19].

EXPERIMENTAL:

Material and Chemicals: Atorvastatin calcium, Amlodipine besilate were obtained as a souvenir samples from Micro Laboratories Ltd., India. The HPLC grade Water, Methanol, Acetonitrile and ortho phosphoric acid were purchased from Merck (Darmstadt, Germany). All reagents used were of HPLC grade except ortho phosphoric acid which was Analytical grade (Rankem).

Instrumentation and Chromatographic Conditions: The HPLC analysis was carried out on a LC system consisted of a model 1100 series liquid chromatography equipped with a binary pump, a vacuum degasser, a variable wavelength programmable UV/VIS detector, a thermostatted column compartment and an thermostatted auto sampler, a Agilent Chemstation software, all from Agilent technologies (Pale Alto, CA, USA). All chromatographic experiments were carried out on an isocratic mode HPLC system (Agilent 1100 series). A Grace smart RP C_{18} (250*4.6) column and phosphate buffer: acetonitrile: methanol in the ratio of 53:43:4 v/v as mobile phase at a flow rate of 1 ml/min and UV detection at 246 nm was used.

Preparation of Standard Solutions: Weigh accurately about 50.0 mg of Atorvastatin calcium working standard and 25.0 mg of Amlodipine besilate working standard in a 100 ml volumetric

flask. Add 50 ml of mobile phase and mix well, then make up to the final volume. Further dilution was made by pipetting 10 ml of mother liquor into 50 ml volumetric flask and make up to the volume with mobile phase. The final conc. of Atorvastatin calcium and Amlodipine besilate were 100 μg/ml, 50 μg/ml respectively. All solutions were stored at 4˚C. Inject the preparation in to the chromatograph and record the peak responses.

Sample Preparation: Weigh accurately about 200mg of powdered tablets in to 100 ml volumetric standard flask and add 50 ml of mobile phase and sonicated for 30 minutes. Then make up the final volume with mobile phase. Shake well and filter the solution. From the above filtrate, pipette out six equivalent volumes in to a different vials. The final conc. of Atorvastatin calcium and Amlodipine besilate were equivalent to the standard solution. All solutions were stored at 4˚C. Inject the preparation in to the chromatograph and record the peak responses.

Chromatographic Procedure: Chromatographic Conditions: A GRACE SMART reversed phase C-18 column mobile phase comprising a mixture of phosphate buffer (1ml ortho phosphoric acid in 1000 ml 0f water) acetonitrile and methanol (53:43:4, v/v) was used. Mobile phase flow rate was maintained at 1 ml/min and eluent were monitored at 246 nm. A 20 [micro] l of sample was injected using a fixed loop, and the total run time was 15 min. All the chromatographic separations were carried out at temperature 50˚C.

Calibration Curves for AML and ATV: Weigh accurately about 50.0 mg of Atorvastatin calcium working standard and 25.0 mg of Amlodipine besilate working standard in a 100 ml volumetric flask. Add 50 ml of mobile phase and mix well, then make up to the final volume. Pipette out 10 ml of the solution into a 50 ml volumetric flask and make up to the volume with mobile phase to obtain final concentrations of 40, 45, 50, 55, 60 μg/ml of AML and 80, 90, 100, 110, and 120 μg/ml of ATV, respectively. Inject the preparation in to the chromatograph and record the peak responses. Calibration curves were constructed by plotting peak area versus concentrations of the drug and regression equations were computed for ATV and AML.

Analysis of Marketed Formulations: Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10 mg ATV (and 5.0 mg of AML) was taken in 100 ml volumetric flask. Add 50 ml of mobile phase and mix well; the flask was sonicated for 5 minutes. The solution was filtered in another 50 ml volumetric flask using Whatman filter paper (No.1) and volume was made up to the mark with the same solvent. From the above filtrate, pipette out six equivalent volumes in to a different vials. The final conc. of Atorvastatin calcium and Amlodipine besilate were equivalent to the standard solution. Inject the preparation in to the chromatograph and record the peak responses. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

Method Validation: The method was validated for accuracy, precision, specificity, detection limit, quantization limit and robustness. The accuracy of the method was determined by calculating recoveries of AML and ATV by method of standard additions. Known amount of AML (0, 4, 5, 6 mg/ml) and ATV (0, 8, 10, 12mg/ml) were added to a pre-quantified sample solutions and the amount of AML and ATV were estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

RESULTS AND DISCUSSION: For simultaneous estimation of Amlodipine and Atorvastatin were preliminary trials were carried out in different mobile phase compositions of phosphate buffer: acetonitrile: methanol in the ratio of 51:45:4, 53:43:4, 55:41:4v/v. The mobile phase consisting phosphate buffer: acetonitrile: methanol (53:43:4, v/v/) was found to be optimal for obtaining well defined and resolved peaks with the mean retention time for Amlodipine and Atorvastatin was found to be 3.337 and 6.067 respectively. The calibration curve was found to be linear over the range of 40-60 µg/ml for AML and 80-120 µg/ml for ATV.

The accuracy of the method was determined by calculating recoveries of AML and ATV by method of standard additions. The recoveries obtained were 99.05-99.63% for AML and 99.85-100.52% for ATV, respectively. The high values indicate that the method is accurate. Instrument precision was determined by performing injection repeatability test and the RSD values for AML and ATV were found to be 0.0581% and 0.0264%, respectively. The intra-day and inter-day precision studies were carried out. For the intra-day study RSD values were found to be 0.0521-0.473% for AML and 0.0261-0.825% for ATV and for inter-day precision study RSD values were found to be 0.0736- 1.16% for AML and 0.06-1.34% for ATV, respectively. The low RSD values indicate that the method is precise. The above data shows that a nanogram quantity of both the drugs can be accurately and precisely determined.

Linearity: Several aliquots of standard solution of AML and ATV were taken in different volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of AML and ATV is between 40-120 µg/ml. Evaluation of two drugs were performed with PDA detector chromatogram extracted at 246 nm, peak area recorded for all the peaks and are given in **table 1**.

TABLE 1: LINEARITY DATA FOR AMLODIPINE AND ATORVASTATIN

Amlodipine		Atorvastatin	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
40	1191.08	80	3625.24
45	1353.00	90	4107.47
50	1482.05	100	4498.91
55	1632.08	110	5019.55
60	1789.84	120	5405.09

The results show the correlation exists between peak area and concentration of drugs within the concentration range (NLT 0.0995) [**Fig 3, 4**].

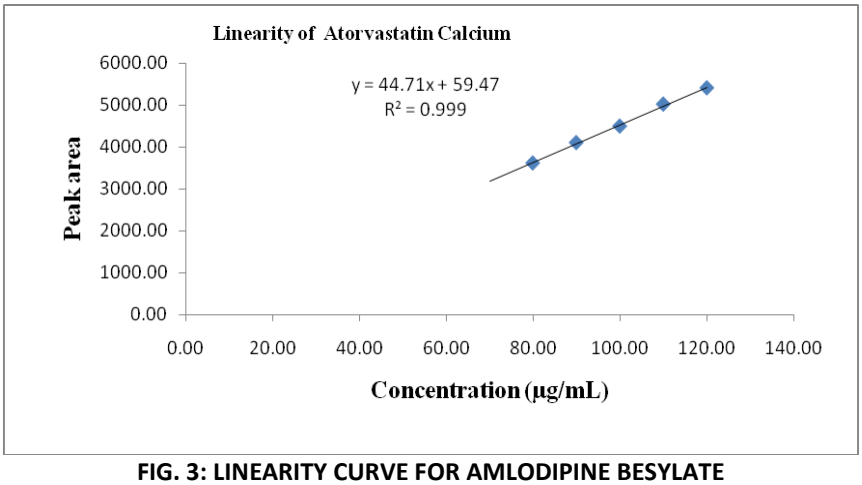

FIG. 3: LINEARITY CURVE FOR AMLODIPINE BESYLATE

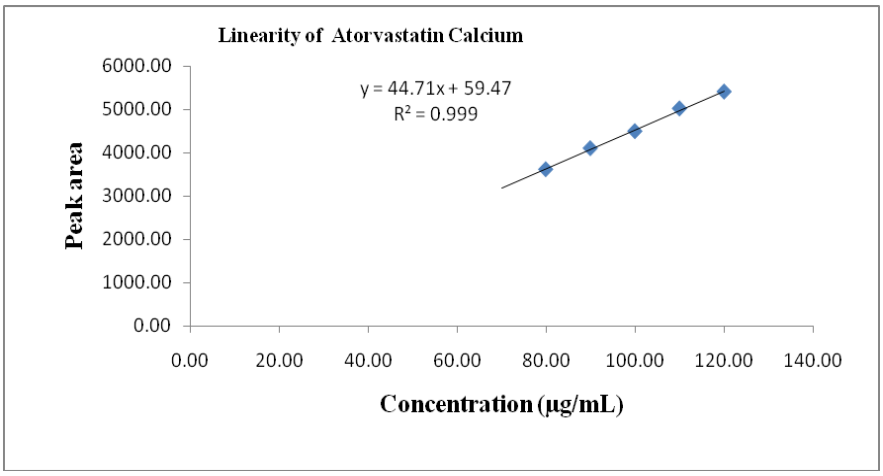

FIG. 4: LINEARITY CURVE FOR ATORVASTATIN CALCIUM

Accuracy: To ascertain the accuracy of proposed method, the accuracy of the method was determined by calculating recoveries of AML and ATV by method of standard additions. The recoveries obtained were 99.60-100.02% for AML and 99.05-100.52% for ATV, respectively. The results are shown in **table 2**.

System Precision: The precision of the method was demonstrated by intraday and inter day variations studies. In the intraday and inter day studies, three repeated injections of standard solutions were made and results were calculated. The results are reported in terms of percentage relative standard deviation (%RSD) 0.0581 for Amlodipine and 0.0264 for Atorvastatin [**Fig. 5**].

TABLE 2: RECOVERY STUDY DATA'S FOR AMLODIPINE AND ATORVASTATIN

	Amlodipine		Atorvastatin	
Added (%)	Recovered ± RSD (%)	Recovered (%)	Recovered ± RSD (%)	Recovered (%)
80	0.0302	99.60	0.0903	99.05
100	0.0417	100.02	0.0211	100.52
120	0.0405	99.63	0.042	99.75

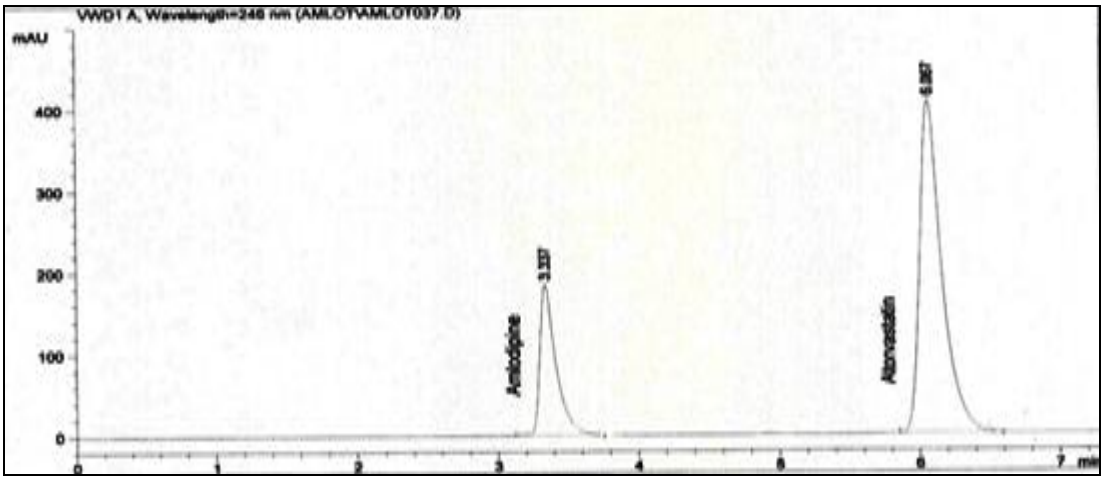

FIG.5: CHROMATOGRAM OF SYSTEM PRECISION

Method Precision: Method precision was carried out by three repeated injections of sample solutions was made and percentage relative standard deviations were 0.0581 for Amlodipine and 0.0264 for Atorvastatin. The results of precision were given in **table 3** and **Fig. 6.**

TABLE 3: METHOD PRECISION DATA FOR AMLODIPINE AND ATORVASTATIN

Drug	Mean Area	SD	RSD (%)
Amlodipine	2324.522	8.5968	0.3698
Atorvastatin	5302.322	26.069	0.4916

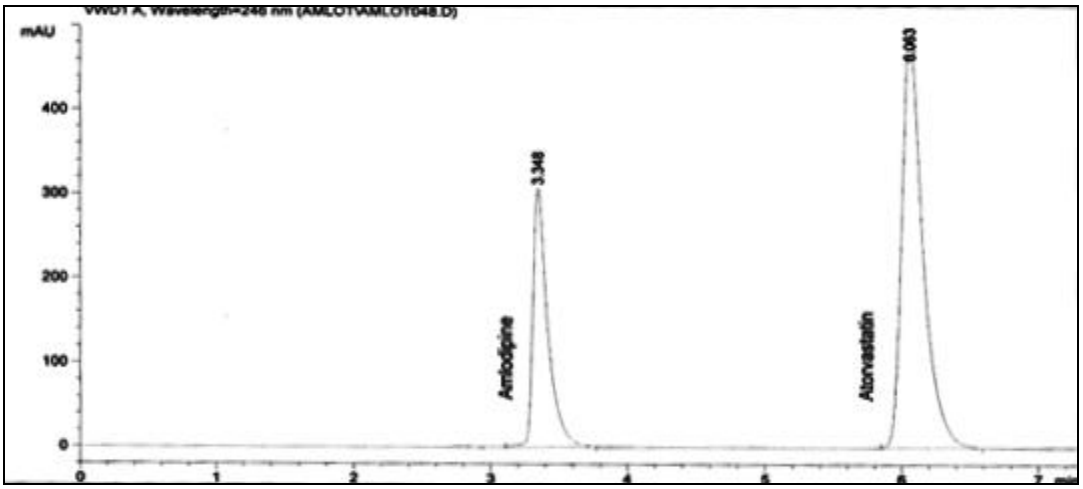

FIG. 6: CHROMATOGRAM OF METHOD PRECISION

Specificity: The specificity was estimated by spiking commonly used excipient (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

System Suitability Parameters: For system precision, five replicate injections of mixed standard solutions were injected and parameters such as the theoretical plate, tailing factor of the peaks were calibrated. Robustness of the method was studied by changing the flow rate of the mobile phase from 1 ml/min to 0.8 ml/min and 1.2 ml/min. Using 1.2 ml/min flow rate, retention time for AML and ATV were observed to be 2.784 and 5.148 min, respectively and with 0.8 ml/min flow rate, retention times for AML and ATV were observed to be 4.184 and 7.736 min, respectively without affecting the resolution of the drugs. When mobile phase composition was changed to Phosphate buffer: acetonitrile: methanol (51:45:4, v/v/v) by increasing percentage of acetonitrile, the retention time for AML and ATV were observed to be 2.784 and 5.148 min, respectively.

CONCLUSION: This study represents the first previously not reported validated method for the simultaneous estimation of Amlodipine and Atorvastatin by RP- HPLC with PDA detector extracted at 246 nm. The proposed method was found to be simple, precise, accurate and rapid for determination of Amlodipine and Atorvastatin in tablet dosage form. The mobile phase is simple to prepare and economical. This analytical method enables quantification of the Amlodipine and Atorvastatin in a range from 40 - 120 µg/ml, with acceptable precision, accuracy, linearity, inter day and intraday precision. This method was validated as per ICH guidelines. The proposed method for simultaneous estimation of

ATVC and AMLB dosage forms were found to be simple, accurate, economical and rapid.

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