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Simultaneous Determination of Atorvastatin Calcium and Amlodipine Besylate from Pharmaceutical Formulation by Reversed Phase High Performance Liquid Chromatography

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A simple, fast and sensitive reverse phase high performance liquid chromatographic method has been developed for the determination of atorvastatin calcium and amlodipine besylate by isocratic elution in its dosage form. These drugs were then chromatographed on a Thermoquest ODS-3 μm column, 250 × 4.6 mm and the detection was carried out at 246 nm. The method was statistically validated for its linearity, accuracy and precision. The intra-day and inter-day variation was found to be less than 0.5% showing high precision of the assay method. The calibration curve was linear in the concentration range 50 to 150% of 200 ppm. Due to its specificity, accuracy/recovery and precision, the proposed method may be used for the determination of atorvastatin calcium and amlodipine besylate in its finished dosage form.

Key Words: High performance liquid chromatography, ODS-octyl decyl silane.

INTRODUCTION

Formulation of atorvastatin calcium and amlodipine besylate is a combination of a synthetic lipid lowering agent and calcium channel blocker, which is taken orally to treat high blood pressure, high cholesterol and chest pain. Atorvastatin calcium **(I)** is chemically described as (βR, δR)2-(flourophenyl)methyl]-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid, calcium salt (2 : 1) trihydrate; $(C_{66}H_{68}Ca_2F_2N_4O_{10})$[1]. Its molecular weight is 1209.4. It is very slightly soluble in water and ethanol but freely soluble in methanol.

Amlodipine besylate **(II)** is 2-[(2-aminoethoxy)methyl]-4-(*o*-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzene sulphonate; $(C_{26}H_{31}ClN_2O_8S)$[1]. Its molecular weight is 567.1. It is a white or almost white crystalline powder, slightly soluble in water, sparingly soluble in ethanol but freely soluble in methanol. It is used in the management of hypertension and angina pectoris.

Amlodipine besylate (**II**) is official in EP[3]. The HPLC method for simultaneous determination of amlodipine besylate with various other combinations (ramipril[5], atenolol[6, 7], enlapril maleate[8] and nifedipine[9]) in pharmaceutical formulations for treatment of cardiovascular system is reported. However, simultaneous determination HPLC method for analysis of **I** and **II** is not reported.

In the present work a simple, rapid and accurate HPLC method for the simultaneous determination of atorvastatin calcium and amlodipine besylate has been reported.

EXPERIMENTAL

Working standards of amlodipine besylate and atorvastatin calcium were obtained from well reputed research laboratories. The purities of these standards were 99.1 and 98.9% respectively. HPLC grade acetonitrile (JT Baker), AR grade orthophosphoric acid and triethylamine were procured from the market. The LC system (Agilent), consisted of binary pump solvent delivery system, a Rheodyne injector (7725i) fitted with a 20 μL loop, column oven and variable wavelength detector. The output signal was monitored and processed using HP Chemstation software.

Standard Solutions

About 25 mg of amlodipine besylate standard (purity 99.1%) and 25 mg of atorvastatin calcium standard (purity 98.9%) were accurately weighed and transferred to a 25 mL volumetric flask. The mixture was then dissolved in diluent to give standard stock solution of 1 mg/mL.

Chromatographic Conditions

Flow rate: 1.0 mL/min; detection 246 nm; injection quantity: 20 μL; column used: Thermoquest -ODS 3 V, 25 cm × 4.0 mm – 3m μm; column temperature: ambient; mobile phase: acetonitrile : buffer (600: 400); buffer solution: add 7.0 mL of 85% ortho-phosphoric acid in 1000 mL of water; adjust the pH to 3.0 ± 0.05 with triethyamine solution; diluent: mobile phase.

Method Development

The aim of this work was to separate amlodipine besylate and atorvastatin calcium using reverse phase chromatographic condition. Various experiments were conducted to select the best stationary and mobile phase that would give optimum resolution and selectivity for the two compounds. The system suitability results are presented in Table-1:

TABLE-1
SYSTEM-SUITABILITY REPORT

Component	Retention time	Relative retention time	Resolution	USP tailing
Atorvastatin calcium	4.21	1.0	—	0.8
Amlodipine besylate	7.02	1.7	8.5	0.7

Calculation

The assay value for atorvastatin calcium and amlodipine besylate were calculated for the formulation using the following formula:

$$\frac{Area_{spl}}{Area_{std}} \times \frac{Wt_{std}}{Wt_{spl}} \times \frac{D}{L} \times \text{purity of std.}$$

where $Area_{spl}$ = peak area response of main peak in chromatogram of sample preparation; $area_{std}$ = peak area response of main peak in chromatogram of standard preparation; Wt_{std} = weight of the standard in mg; Wt_{spl} = weight of the sample in mg; D = dilution factor; L = label claim of drug dosage (in mg); purity of std % = purity of standard on as is basis.

Method Validation

Method Selectivity: The individual components were injected one by one and the respective chromatographs were recorded. Fig. 1 represents an ideal chromatograph in which all the components are well separated from each other.

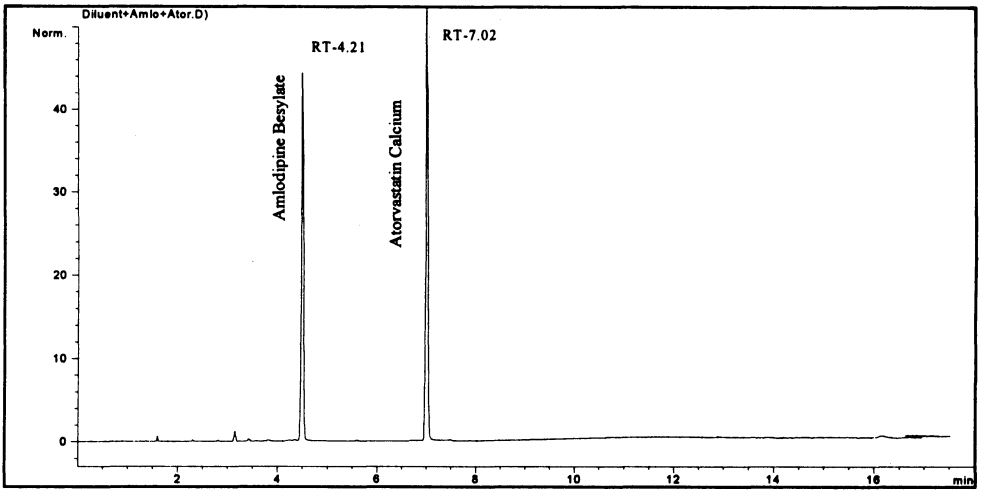

Fig. 1. A typical chromatogram representing separation of amlodipine besylate and atorvastatin calcium

Method Linearity: This experiment is carried out to demonstrate that the response obtained with amlodipine besylate and atorvastatin calcium is linear over a specified range of concentration, *i.e.*, any minor increase or decrease in the concentration of amlodipine besylate and atorvastatin calcium in the sample will be reflected by proportional increase or decrease in the detector response or in the areas under corresponding main peak. The system was studied for its ability to precisely detect the range of concentration of amlodipine besylate and atorvastatin calcium. Five linearity levels for amlodipine besylate and atorvastatin calcium were prepared by diluting standard stock solution as given in Table-2.

TABLE-2
DILUTION OF STANDARD SOLUTION FOR LINEARITY STANDARD

Linearity levels (ppm)	Volume of std. stock solution added in mL (both amlodipine besylate + atorvastatin calcium)	Volume made up to (in mL)
100	5.0	50
150	7.5	50
200	10.0	50
250	12.5	50
300	15.0	50

The plot ratio of area of amlodipine besylate and atorvastatin calcium *vs.* respective concentrations (in ppm) for both drugs are found to be linear in the range of 100 ppm to 300 ppm with coefficient of correlation (0.999 to 1.0) (Tables 3 and 4). The intercept value was found to be not significantly deficient than zero.

TABLE-3
LINEARITY STUDY

Concentration level (in ppm)	Area obtained due to atorvastatin calcium	Area obtained due to amlodipine besylate
100	123473	45236
150	175000	67887
200	249562	90478
250	312511	113102
300	373214	135762

TABLE-4
LINEARITY REGRESSION ANALYSIS

Parameter	Atorvastatin calcium	Amlodipine besylate
Equation of the least square line	Y = 1273.99x – 8045.20	Y = 452.53x – 184.00
Slope of the line	1273.99	452.53
Y-intercept at the line	–8045.20	–13.80
Correlation coefficient	0.999	1.000

Method Accuracy and Recovery

This is performed by measuring the accuracy of the method reported as percentage recovery by adding varying amounts of amlodipine besylate and atorvastatin calcium solution to a known weight of amlodipine besylate and

atorvastatin calcium stock solution. The values of the recovery experiment were presented in tabular form indicating added amount, amount recovered and percentage recovery (Tables 5 and 6). The table is prepared containing mean percentage recovery, standard deviation of percentage recovery and relative standard deviation of percentage recovery.

TABLE-5
ACCURACY/RECOVERY OF AMLODIPINE BESYLATE

Concentration level (in ppm) added externally	Area observed due to amlodipine besylate standard solution	Area obtained due to 200 ppm of amlodipine besylate	Area observed due to added amlodipine besylate	Percentage accuracy (in %)
20	9008	90478	99502 – 9008 = 90494	100.02
40	18064	90478	108827 – 18064 = 90763	100.31
60	27143	90478	117628 – 27143 = 90485	100.01

Mean 100.11; standard deviation 0.1704; Relative standard deviation 0.17.

TABLE-6
ACCURACY/RECOVERY OF ATORVASTATIN CALCIUM

Concentration level (in ppm) added externally	Area observed due to atorvastatin calcium standard solution	Area obtained due to 200 ppm of atorvastatin calcium	Area observed due to added atorvastatin calcium	Percentage accuracy (in %)
20	24677	249562	275215 – 24677 = 250538	100.39
40	49455	249562	300086 – 49455 = 250631	100.43
60	73732	249562	323184 – 73732 = 249452	99.96

Mean 100.26; standard deviation 0.2606; Relative standard deviation 0.26.

Method Precision

To demonstrate the agreement among results when a series of measurements are done with atorvastatin calcium and amlodipine besylate sample, three injections of three different concentrations were injected onto the chromatographic system on the same day and the values of relative standard deviation were calculated to determine the intra-day precision. The same study was repeated after 1 week to determine inter-day precision. Individual results are listed in tabular form (Table-7).

TABLE-7
REPEATABILITY RESULTS

Observation No.	Amlodipine besylate			Atorvastatin calcium		
	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
Inter Day Precision (Day-1)						
1	45218	90482	135725	124824	249456	374185
2	45198	90469	134895	124729	249569	373985
3	45239	90435	135028	124759	249558	374058
Mean (n = 3)	45218	90462	135216	124771	249528	374076
Std. Dev	20.5	24.3	445.8	48.6	62.3	101.2
RSD	0.05	0.03	0.33	0.04	0.03	0.03
Inter-Day Precision (after 7 days)						
1	45252	90492	135278	124799	249389	374885
2	45298	90519	134985	124785	249725	374905
3	45279	90486	135701	124762	249627	374798
Mean (n = 3)	45276	90499	135321	124782	249580	374863
Std. Dev	23.1	17.6	360.0	18.7	172.8	56.9
RSD	0.05	0.02	0.27	0.02	0.07	0.02
*Intra-Day Precision**						
Mean (n = 6)	45247	90481	135269	124776	249554	374469
Std. Dev	37.3	27.7	366.9	33.5	119.7	43.7
RSD	0.08	0.03	0.27	0.03	0.05	0.12

*For the calculation of intra-day precision the areas of all three replicate injections are taken from both inter-day precision procedures.

RESULTS AND DISCUSSION

The regression value was found to be 1.0 for amlodipine besylate and atorvastatin calcium, which shows that the response is linear from 100 ppm to 300 ppm. Selectivity experiment showed that there is no interference or overlapping of peaks either due to exipients or diluents with the main peak of atorvastatin calcium and amlodipine besylate. The selectivity of the method is proved with the very high value of resolution for amlodipine besylate and atorvastatin calcium. High percentage of recovery shows that the method is free from interference of amlodipine besylate from atorvastatin calcium. The RSD values for both intra-day precision study and inter-day precision are less than 0.5%, which confirm that the method is sufficiently precise. The proposed method is simple, fast, accurate and precise. Thus, the proposed method can be used for the routine analysis in quality control of amlodipine besylate and atorvastatin calcium.

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