RP-HPLC Method for the Simultaneous Determination of Atorvastatin and Amlodipine in Tablet Dosage Form

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A simple, precise, accurate, and rapid HPLC method has been developed, and validated for the determination of atorvastatin and amlodipine simultaneously, in combined tablet dosage form. The mobile phase used was a mixture of acetonitrile and 0.03M phosphate buffer pH 2.9 (55:45% v/v). The detection of atorvastatin and amlodipine was carried out on dual γ absorbance detector at 240 nm and 362 nm, respectively. Results of the analysis were validated statistically, and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.

Atorvastatin calcium[1], chemically, 1H-pyrrole-1-heptanoic acid, [R-(R*,R*)]-2-(4-flurophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-calcium salt (2:1), is an antihyperlipoproteinemic drug[2,3], used for treatment of hypercholesterolemia. 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] Literature survey reveals that analytical methods,[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 (ATS) 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 The proposed method is rapid, simple, accurate, and reproducible, and can be successfully employed in the routine analysis of both these drugs simultaneously, in tablet dosage form.

Separation was carried out on an isocratic HPLC system (Waters), with Waters 1525 binary HPLC pump, Waters 2487 UV dual λ absorbance detector, Waters Breeze software, and RP-C_{18} column (150x4.6 mm I.D.; particle size 5 μm). The chromatographic estimation was performed using the following conditions: the mobile phase used was acetonitrile and 0.03 M phosphate buffer

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pH 2.9, in the ratio of 55:45% v/v. The run time and the flow rates were 6 min and 1 ml/min, respectively. Detection wavelengths of ATS and AML were set at 240 nm and 362 nm, respectively. Standard stock solutions of 1 mg/ml of ATS and 1 mg/ml of AML were prepared in methanol, and used for estimation. For construction of calibration graph, stock solutions were further diluted with mobile phase ranging from 0.1-20 μg/ml. This method was applied to determine ATS and AML, in two different market samples. For analysis of tablet formulation, an accurately weighed tablet powder equivalent to 10 mg of ATS and 5 mg of AML, was taken in a volumetric flask (25 ml). The powder was dissolved in 15ml methanol, shaken thoroughly, and made upto the volume with methanol. Then the solution was filtered through Whatman filter paper (No. 41) and further diluted with distilled water, to get the concentrations of 10 μg/ml and 5 μg/ml of atorvastatin and amlodipine, respectively. These solutions were injected, and the chromatograms were recorded.

The method was validated interms of linearity, accuracy, inter-day and intra-day precision, reproducibility, and specificity. The limit of detection (LOD) and limit of quantitation (LOQ), were also determined. The accuracy of the method was evaluated by carrying out recovery studies. For that, known concentration of standard solutions were added to the pre-analysed sample solution, and the recovery was calculated. The intra-day precision was determined by analysing standard solutions in the linearity range of calibration curve in triplicate on the same day, while inter-day precision was determined by analysing corresponding standard solutions daily, for a period of one week. The RSD or CV of <2.5% was observed. The validated data was furnished in Table 1.

Both atorvastatin and amlodipine are soluble in methanol, therefore methanol was selected as solvent. The mixture of acetonitrile and 0.03M phosphate buffer pH 2.9 in the ratio of 55:45% v/v, could resolve atorvastatin and amlodipine, with better resolution. On the other hand, 1 ml of triethyl amine helped in sharpening of the peaks. The retention times of amlodipine and atorvastatin are 1.59 min and 5.12 min, respectively (Fig. 1). Linearity range for atorvastatin and amlodipine were 0.2-20 μg/ml (r=0.999) and 0.1-20 μg/ml (r=0.9999), respectively. The linear regression equations are Y=-30.905+52814.6X for atorvastatin, and Y=173.76+46735.6X for amlodipine. The high percentage of recovery of the drugs, indicate that the method is highly accurate. The content of and the percentage of drugs in two different market samples (Table 2), indicate that the proposed method is simple, rapid, precise, and accurate, for the estimation of atorvastatin and amlodipine, in its pharmaceutical formulation.

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TABLE 1: VALIDATION SUMMARY

System Suitability	Results	
	Atorvastatin	Amlodipine
Theoritical Plates (N)	9000	3000
Resolution	2.06	-
Linearity range (μg/ml)	0.2-20	0.1-20
Percentage recovery % (Accuracy)	100.37	98.92
LOD (μg/ml)	0.02	0.01
LOQ (μg/ml)	0.06	0.03
Tailing factor	1.09	1.00
Capacity factor	4.86	0.99
Symmetry factor	1.09	1.00

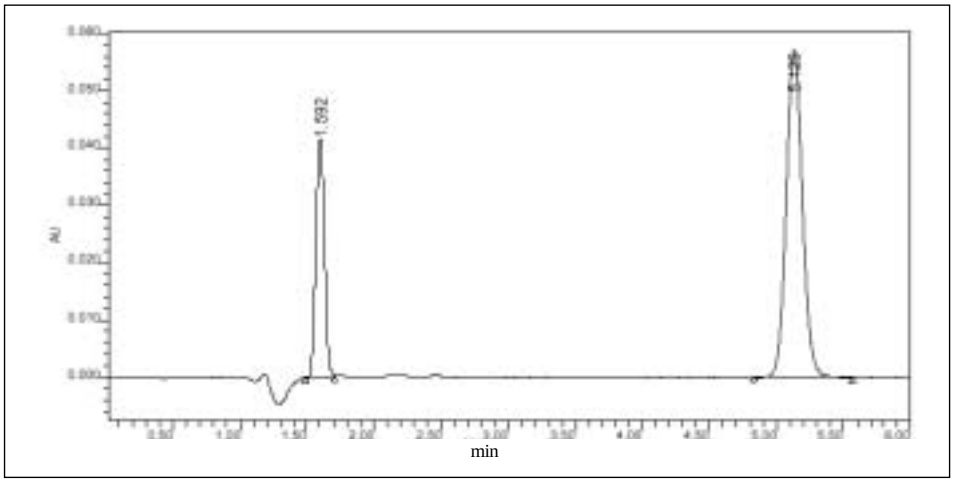

Fig. 1: Typical chromatogram of atorvastatin and amlodipine

TABLE 2: ASSAY AND RECOVERY STUDIES

Formulation#	Drug	Label claim (mg)	% of amount found*	Amount of standard added (mg)	Amount recovered*	% recovery
Tablet 1	Atorvastatin	10	99.14	10	10.02	100.34
	Amlodipine	5	100.05	5	5.01	101.37
Tablet 2	Atorvastatin	10	99.91	10	9.98	98.06
	Amlodipine	2.5	98.09	2.5	2.42	98.92

*Mean of five determinations. #The commercial preparations used were; Tablet 1: Lipitas, INTAS Pharmaceuticals Ltd. Tablet 2: Am At, Nicholas Piramil India Ltd.

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