Research Article

Simultaneous Estimation of Atorvastatin and Amlodipine Besilate in Pharmaceutical Formulation by A Novel HPLC Method

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ABSTRACT
A rapid and sensitive high performance liquid chromatography method for determination of Atorvastatin and Amlodipine Besilate has been developed. The chromatography system used a reversed phase C-18 column with UV- Vis detection at 240 nm. Mobile phase consisted of acetonitrile : ammonium dihydrogen orthophosphate (40:60 v/v) (pH adjusted to 3.0 using 10% ortho phosphoric acid) at a flow rate of 1.0 ml/min. The calibration curve was linear in the concentration range of 2.5-12.5 µg/ml for Amlodipine Besilate 5-25 µg/ml for Atorvastatin. The lower limit of detection was found to be 10µg and 15µg for Amlodipine Besilate and Atorvastatin respectively.

Keywords: Amlodipine Besilate,Atorvastatin RP-HPLC analysis,C-18 column.

INTRODUCTION

Amlodipine Besylate is a dihydropyridine calcium antagonist used in the treatment of hypertension and angina pectoris[1]. It inhibits transmembrane influx of calcium ions into cardiac and smooth muscles[2]. Atorvastatin is a antihyperlipidemic drug. It acts by inhibiting HMGCoA reductase enzyme involved in cholesterol synthesis[3]. It reduces LDL-cholestrol,triglycerides and increases HDL-cholesterol[4,5].

The objective of the present work was to develop and validate the rapid and sensitive high-performance liquid chromatography (HPLC) method for simultaneous determination of Atorvastatin and Amlodipine Besilate in tablets.

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Materials and Methods

Drugs

Amlodipine Besylate and Atorvastatin.Trade drug product (Mankind).

Chemicals and solvents

Triethylamine and orthophosphoric acid were purchased from S.D. Fine Chemicals Ltd., India. Acetonitrile of HPLC grade were purchased from Qualigens Fine Chemicals, India. The gift samples of the drug were received from IndSwift Lab. Ltd. Chandigarh as a gift sample. Nylon syringe membrane filters (0.2 µm) were purchased from Sartoris, Germany.

HPLC system

The HPLC system consisted of a delivery pump (Water 600 pump controller), a reversed phase analytical column C-18 (250 × 4.6 mm) 5 µm (Kromasil), a Rheodyne sample injector with a 20 µl loop volume and a variable wavelength (UV-Vis) detector (waters 2487 Dual Absorbance Detector).

Chromatographic conditions

Mobile phase consisted of acetonitrile : ammonium dihydrogen orthophosphate (40:60 v/v) (pH adjusted to 3.0 using 10% ortho phosphoric acid) at a flow rate of 1.0 ml/min.The solution was filtered through a 0.2 µm membrane filters. The eluent was monitored with a UV-Vis detector set at240 nm with a flow rate of 1.0 ml/min. Mobile phase was stirred on a magnetic stirrer during the HPLC run.

Standard solution and calibration curve:

A standard stock solution of Atorvastatin (500 µg/ml) and Amlodipine Besilate (500 µg/ml) were prepared in water for HPLC. Subsequent dilutions were made in mobile phase to give the concentrations 2.5,5,6.25,10 and12.5 µg/ml for

Amlodipine Besilate and5,10,12.5,20 and 25 µg/ml for Atorvastatin. The calibration curve was obtained by plotting the ratio of peak height of drug versus concentration.

Assay

Twenty tablets were weighed accurately and finely powdered. The powder equivalent to 72.6 mg of Atorvastatin and 104.4 mg of Amlodipine Besilate was weighed accurately and dissolved in 100 ml water for HPLC. The solution was filtered through 0.2 µm membrane filter paper. Twenty µl of this solution was injected in triplicate under the specified conditions. The peak height of drug obtained were related to slops and intercepts from the calibration data to calculate concentration of the drugs

Table I: Results of HPLC assay

Atorvastatin		Amlodipine Besilate	
Amt. Claimed mg/tablet	Amt. found mg/tablet	Amt. Claimed mg/tablet	Amt. found mg/tablet
5.0	5.02 4.98 5.06	60.0	60.01 59.09 60.05
Mean	5.01		59.82
RSD	0.77		0.94

Validation of the assay

To study the accuracy, reproducibility and precision, recovery experiments were carried out.

The recovery of the added standard was studied at three different levels. To an aliquot of the analyzed formulation a known concentration of standard solution was added. The content of Atorvastatin and Amlodipine Besilate were determined (Table 2). Linear regression analysis was performed to calculate the slope, the intercept and the correlation coefficient (r) of the calibration curve (Table 3).

Table II: Results of recovery studies

Atorvastatin			Amlodipine Besilate		
Amount added(mg)	Amount found(mg)	Percentage Recovery	Amount added(mg)	Amount found(mg)	Percentage Recovery
5	5.02	100.4	5	4.96	99.2
10	10.12	101.2	10	9.87	98.7
12	11.98	99.8	12	11.92	99.3
	Mean	100.46		Mean	99.06

Table III. Linear regression data for calibration curve

Parameter	Amlodipine Besilate	Atorvastatin
Calibration Range(µg/ml)	2.5-12.5	5-25
Theoretical Plates	1418.24	8570.99
Tailing Factor	1.2	0.7
LOD(µg/ml)	0.10	0.15
LOQ(µg/ml)	0.30	0.40

RESULTS AND DISCUSSION

System suitability tests were carried out on freshly prepared standard stock solutions of drugs (Table 4). The calibration curve was linear in the range of 2.5-12.5 µg/ml for Amlodipine Besilate and 5-25 µg/ml for Atorvastatin. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.10 µg and 0.30µg/ml for Amlodipine Besilate and 0.15µg/ml and 0.4 µg/ml for Atorvastatin.

CONCLUSION

In conclusion, our method is rapid, sensitive, reproducible and well suited to the simultaneous determination of Atorvastatin and Amlodipine Besilate by internal standard method.

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