

METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND AMLODIPINE IN COMBINATION IN TABLET DOSAGE FORM BY UV SPECTROSCOPY, USING MULTI-COMPONENT MODE OF ANALYSIS

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Received on: 08-05-2008; Accepted on :22-07-2008

ABSTRACT

Atorvastatin Calcium and Amlodipine Besilate in combination are available as tablet dosage forms in the ratio of 2:1. A simple, reproducible and efficient method for the simultaneous determination of Atorvastatin Calcium and Amlodipine Besilate in tablet dosage form has been developed. The developed method is based on the simultaneous estimation by UV Spectroscopy, using multi-component mode of analysis. 50% v/v aqueous methanol was used as blank. The validation studies were performed according to ICH guidelines.

Key words: UV-Spectroscopy, Multicomponent mode of analysis, Atorvastatin Calcium, Amlodipine Besilate, Validation

INTRODUCTION

Atorvastatin calcium is chemically [R-(R, R*)]-2-(4-fluorophenyl)-β-d-dihydroxy-5 (1-methylethyl)-3-phenyl-4 [(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate (**Fig. 1**)¹. It is inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis. It is not official in I.P., U.S.P. and B.P. till date. Amlodipine Besilate is chemically 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzenesulphonate (**Fig. 2**)¹. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It is official in British Pharmacopoeia² but not in I.P and USP. Review of literature reveals that no method is described for simultaneous estimation of Atorvastatin Calcium and

Amlodipine Besilate by UV-Spectroscopy, using multicomponent mode of analysis. The present paper describes simple, reproducible and sensitive UV-Spectroscopy method for the determination of Atorvastatin Calcium and Amlodipine Besilate in combination in tablet dosage form (10 mg Atorvastatin and 5 mg Amlodipine).

5. MATERIALS AND METHODS:

Instruments:

- (1) UV-Visible spectrophotometer, UV-1601 (Shimadzu)
- (2) Weighing balance, HR 200 (Afcoset)
- (3) Ultra sonic bath, SW 45 (Toshcon/ Tosniwal)

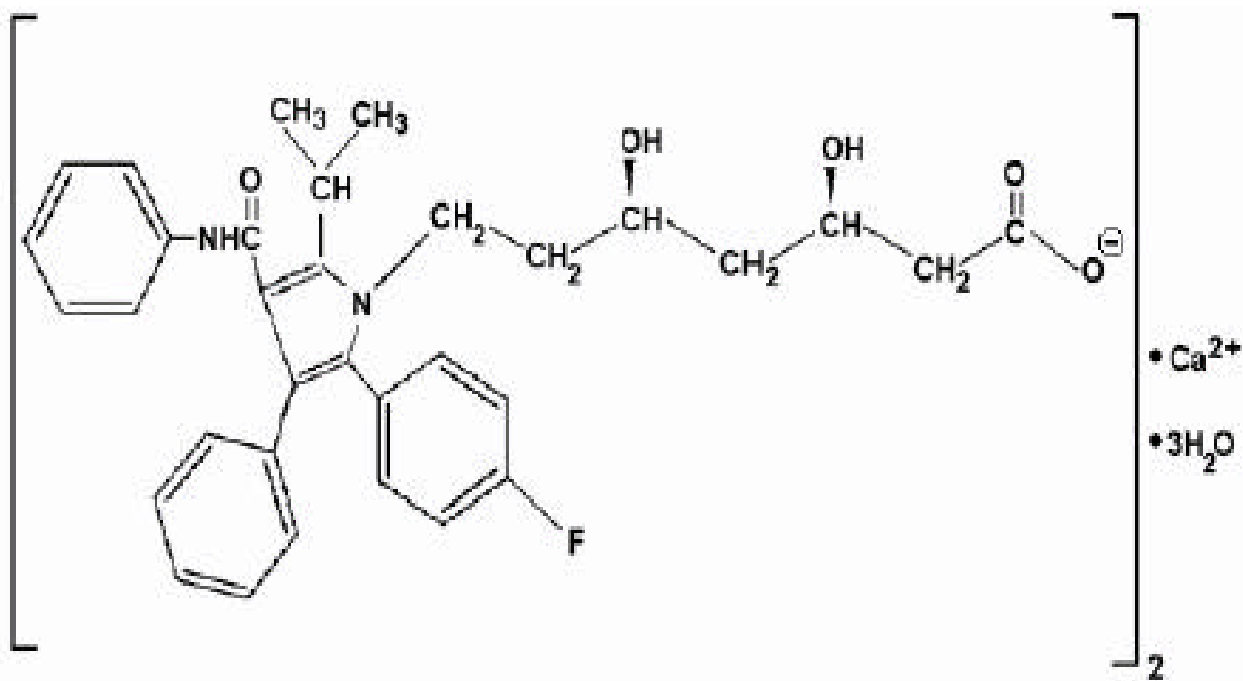
Drug Samples:

Working standard of Atorvastatin Calcium and Amlodipine Besilate were obtained as gift sample from Mankind Pharma, Ltd., Delhi

Chemicals and Reagents:

Methanol AR grade, (Merck). In house produced distilled water was used.

Fig. 1: Chemical structure of Atorvastatin Calcium



METHOD:

Preparation of stock solution of Amlodipine

Amlodipine Besilate equivalent to 25 mg of Amlodipine was accurately weighed and transferred to 250 ml volumetric flask. About 200 ml of 50% v/v aqueous methanol was added and sonicated to dissolve. The volume was made up to the mark with 50% v/v aqueous methanol. The final dilution contained 100µg/ml of Amlodipine.

Preparation of stock solution of Atorvastatin

Atorvastatin calcium equivalent to 20 mg of Atorvastatin was accurately weighed and transferred to a 100 ml volumetric flask. About 90 ml of 50%v/v aqueous methanol was added and sonicated to dissolve. The volume was made up to the mark with 50%v/v aqueous methanol. The final dilution contained 200µg/ml of Atorvastatin.

Preparation of synthetic mixture of Atorvastatin and Amlodipine

10 ml each of the stock solutions of Atorvastatin and Amlodipine were transferred to a 100 ml volumetric flask. The volume was made up to the mark with 50% v/v aqueous methanol. The resultant solution contained 20µg/ml of Atorvastatin and 10µg/ml of Amlodipine.

Linearity and calibration

7, 8, 9, 10, 11, 12, and 13 ml each of the stock solutions of Atorvastatin, 200µg/ml, and Amlodipine, 100µg/ml, were transferred to a series of six 100 ml volumetric flasks. The volume in each flask was adjusted to 100 ml with 50% v/v aqueous methanol and mixed so as to obtain solutions of final concentrations in the range of about 14 to 26µg/ml for Atorvastatin and 7 to 13µg/ml for Amlodipine. These solutions were analysed using multi component mode of analysis. The method was found to be linear in the range of 70 to 130% of the test concentration. In the linearity study, regression equation and correlation coefficient for Atorvastatin and Amlodipine were found to be $y = 0.9924x - 0.0629$, $r = 0.9991$ and $y = 0.9835x + 0.0529$, $r = 0.9993$ respectively.

Recovery studies and validation of the method according to ICH Q2A guidelines³⁻⁴:

The following validation parameters; linearity, range, accuracy, precision and specificity were studied. The recovery of added standard (80%, 100%, and 120%) was found at three same concentration levels for each drug. From the total amount of drug found, the percentage recovery was calculated. Table 1 shows recovery study results of Atorvastatin and Amlodipine. Validation studies were carried out according to the ICH Q2A guidelines and results are shown in Table 2.

Estimation of drugs in tablet dosage forms

Twenty tablets were taken and were crushed to a fine powder. The powder sample equivalent to 20 mg of Atorvastatin and 10 mg of Amlodipine was transferred to a 100 ml volumetric flask and about 80 ml of 50%v/v aqueous methanol was added and sonicated to dissolve. The volume was made up to the mark with 50%v/v aqueous methanol. This solution was filtered through Whatman filter paper 42. 10 ml of this solution was diluted to 100 ml with 50%v/v aqueous methanol. The solutions were analyzed by multicomponent mode of analysis. The samples were analyzed in triplicate. 50%v/v aqueous methanol was used as blank. Figure 3 shows spectrum of Atorvastatin. Figure 4 shows spectrum of Amlodipine. Figure 5 shows overlay spectrum of Atorvastatin, and Amlodipine .

Fig. 2: Chemical structure of Amlodipine Besilate

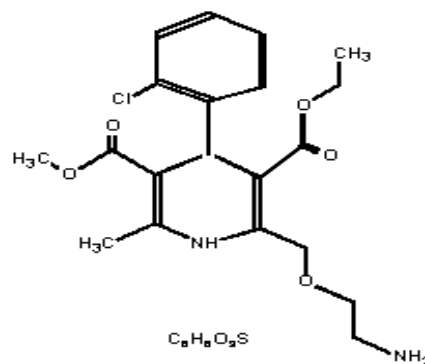


Fig. 3: Spectrum of Atorvastatin

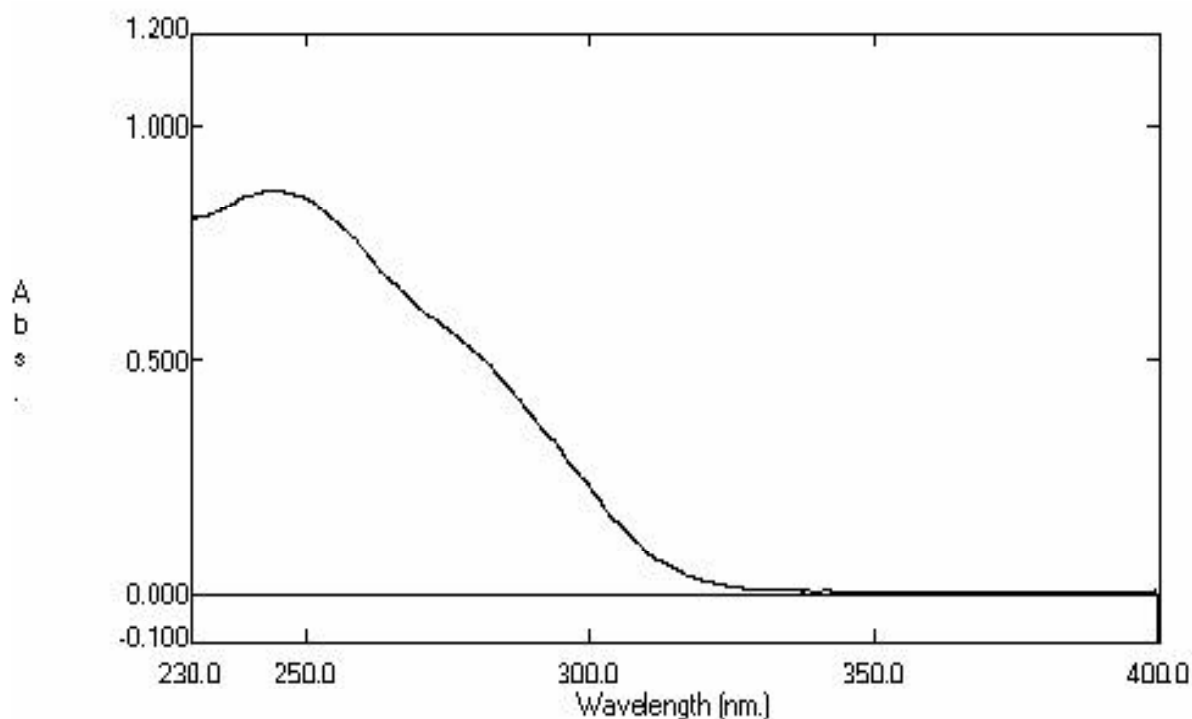


Fig. 4: Spectrum of Amlodipine

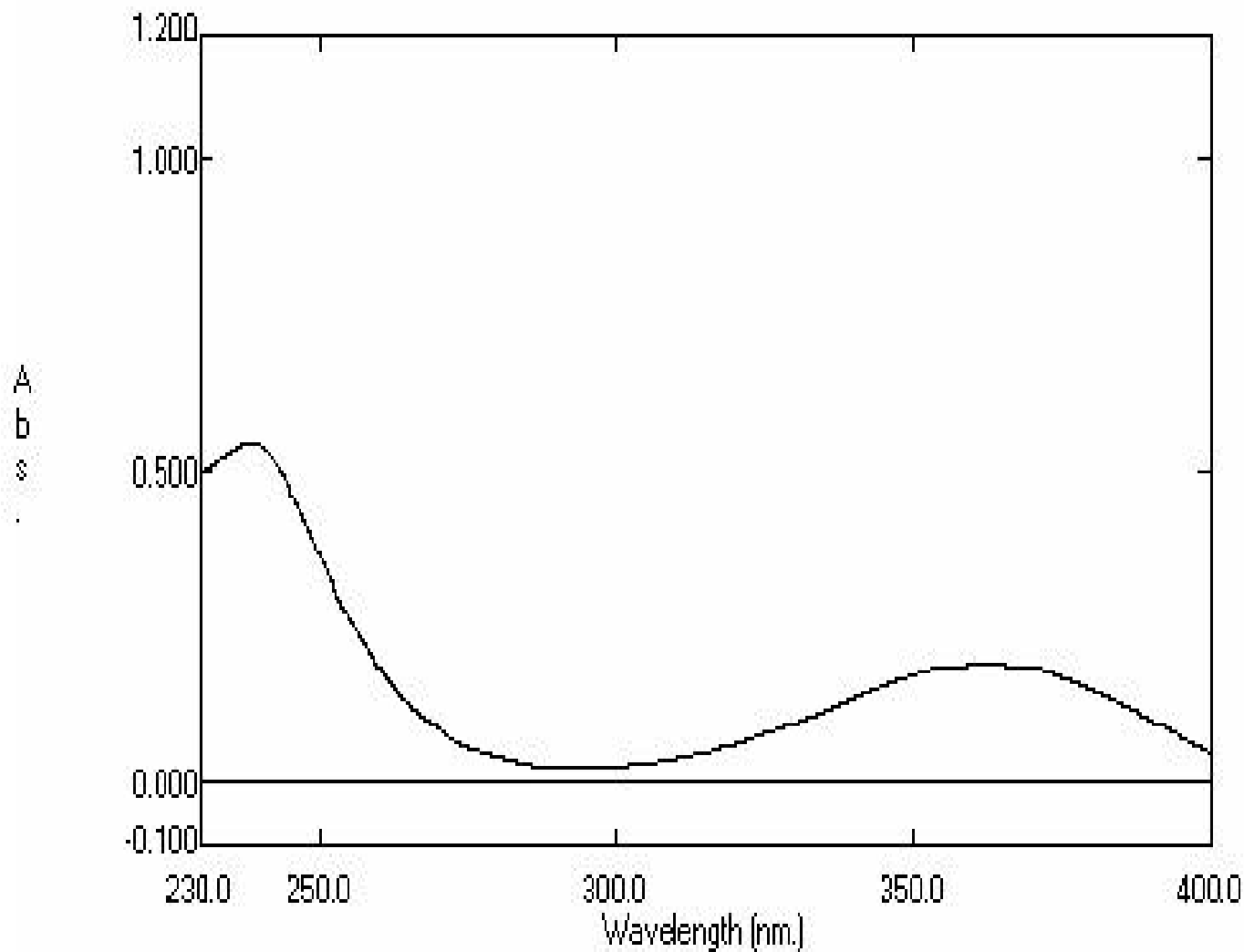


Table 1: Result of Recovery study of Atorvastatin and Amlodipine

S.No	Drug	Conc. before spiking(mg/ml)	Reference std. added(mg/ml)	Conc. after spiking(mg/ml)*	Percentrecovery	Mean percent recovery
1	Atorvastatin	19.715	15.930	35.457	99.04	99.03
		19.715	19.913	39.489	99.30	
		19.715	23.896	43.365	98.76	
2	Amlodipine	9.8034	7.9136	17.585	98.65	98.84
		9.8034	9.8922	19.566	98.68	
		9.8034	11.871	21.594	99.18	

*Mean of triplicate determinations

Fig. 5: Overlay Spectrum of Atorvastatin and Amlodipine

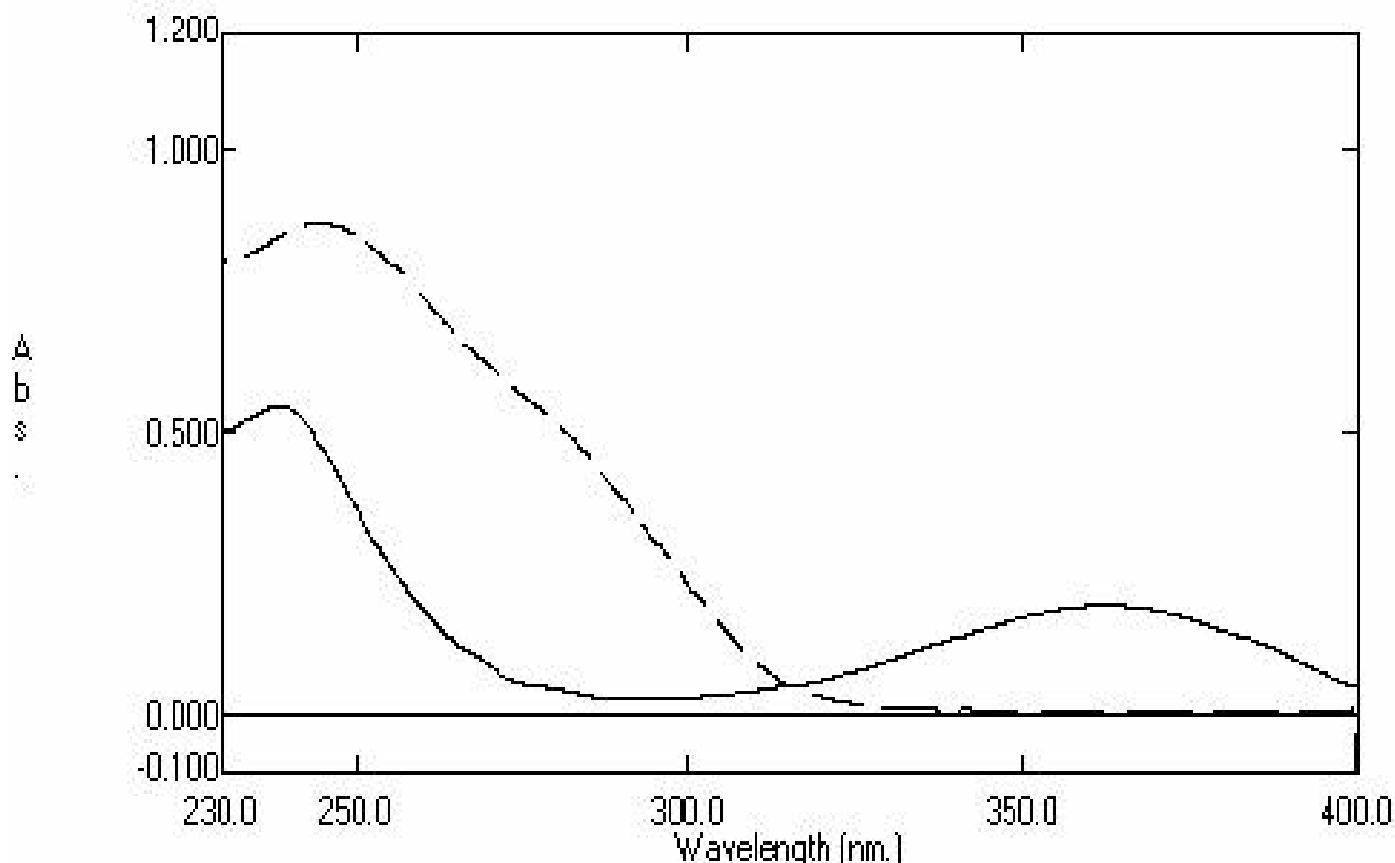


Table 2: Summary of Validation Parameters

PARAMETER	OBSERVATION	
	Atorvastatin	Amlodipine
Specificity	No interference was found w.r.t. excipients	
Linearity (Correlation coefficient r)	0.9991	0.9993
Range	70 to 130%	70 to 130%
Accuracy (% Recovery)	99.03%	98.84%
Precision RSD		
Repeatability (n= 6)	0.898	1.670
Intra-day (n=3)	1.130	0.803
Inter-day (days=3)	0.506	0.721

6. RESULTS AND DISCUSSIONS:

A UV-spectroscopic, multicomponent mode of analysis, method was developed for the simultaneous estimation of Atorvastatin and Amlodipine in tablet dosage forms. Solvent used was 50% v/v aqueous methanol. The absorbance was recorded at 245 and 363 nm. The developed validated method is simple, rapid, precise and accurate. The newly developed method can be used for routine analysis as method for the simultaneous estimation of Atorvastatin and Amlodipine in tablet dosage forms.

7. ACKNOWLEDGEMENTS:

We are sincerely thankful, Deptt of Pharma Science SGRR ITS Dehradun for allowed working at their esteemed premises. also oblige to m/s Mankind Pharma, Ltd. Delhi, for providing working standards of Atorvastatin Calcium and Amlodipine Besilate.

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Source of support: Deptt of Pharma Science SGRR ITS Dehradun , Conflict of interest: None
