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Research Article

NEW SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND ATORVASTATIN CALCIUM IN TABLET DOSAGE FORMS

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ABSTRACT

Two simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of Amlodipine besylate (AMD) and Atorvastatin calcium (ATR) in tablet dosage form have been developed. First method is simultaneous equation method; in this method 361nm and 246 nm were selected to measure the absorbance of drugs at both wavelengths. The second method is Q-value analysis based on measurement of absorptivity at 238.8 nm (as an iso-absorptive point) and 246 nm. AMD and ATR at their respective maximum wavelength 361 nm and 246 nm and at isoabsorptive point 238.8 nm shows linearity in a concentration range of 0.5-30 µg/mL. Recovery studies range from >99.82% for AMD and >98.09% for ATR in case of simultaneous equation method and >100% for AMD and >98.45% for ATR in case of Q-analysis method confirming the accuracy of the proposed method. The proposed methods are recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific (no heating and no organic solvent extraction is required).

Keywords: Amlodipine, Atorvastatin, Simultaneous equation method, Q analysis

INTRODUCTION

Amlodipine is a white crystalline powder which is slightly soluble in water, sparingly soluble in ethanol and freely soluble in methanol. It is official in B.P. Chemically Amlodipine, (Fig 1.) is 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4- dihydro-6-methyl-3, 5-pyridinedicarboxylatebenzenesulfonat[1]. Amlodipine is a dihydropyridine derivative with calcium antagonist activity[2]. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina[3]. Amlodipine acts by inhibiting the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. Atorvastatin is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent[4]. Chemically, Atorvastatin (Fig 2.) is [R-(R*, R*)]-2-(4-flurophenyl)-B, B—dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid[5]. Atorvastatin is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylgultaryl-coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of LDL receptors on the surface of the cells, which inturn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood[6-8].

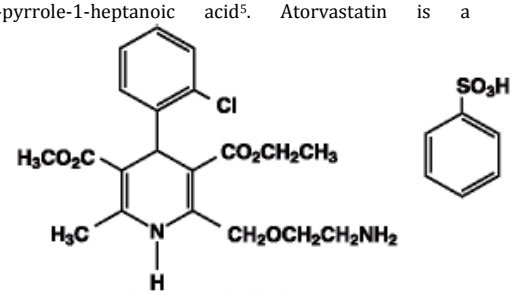

Fig. 1.Amlodipine

Literature survey revealed that no UV methods are reported for the simultaneous determination of Amlodipine and Atorvastatin till date. Methods are available for the quantification of Amlodipine individually and with other combinations other than Atorvastatin by HPLC[9-13], by UV[14-15]. Methods are available for the quantification of Atorvastatin individually and with other combinations other than Amlodipine HPLC [16-18]. Present study involves development and validation of two spectrophotometric methods for the simultaneous determination of Amlodipine and Atorvastatin (ATR) in pharmaceutical formulations and in Drug substances.

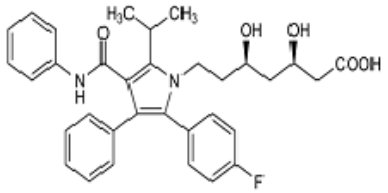

Fig. 2.Atorvastatin

MATERIALS AND METHODS

Instrumentation and chemicals

Spectral runs were made on a Double beam UV-Visible spectrophotometer, model-T80+ was employed with spectral bandwidth of 0.2 nm and automatic wavelength corrections with a pair of 10 mm quartz cells. The software is UVWin5 ver5.1.1, PG instruments. ATR and AMD were purchased from SL drugs & Pharmaceuticals (Hyderabad, India). Purified water was prepared using a Millipore Milli-Q (Nanopure Diamond, Barnstead thermolyne, USA) water purification system. Acetonitrile, Methanol was purchased from Merck Ltd. (Mumbai, India)

Preparation of standard drug solutions

An accurately weighed 10 mg of each of ATR and AMD was dissolved in 10 ml of methanol to obtain a concentration of 1 mg/mL each. From 1 mg/mL solution 1 ml was taken and made to 10 ml with methanol to obtain a concentration of 100µg/mL each. Daily working standard solutions of AMD and ATR was prepared by suitable dilution of the stock solution with methanol.

Determination of maximum wavelength and Iso-absorptive point

By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg /ml of AMD and 10 µg /ml of ATR were scanned separately in the range of 200- 400 nm to

determine the wavelength of maximum absorption for both the drugs. AMD showed absorbance maxima at 361nm (λ1) and 236.5 nm and ATR showed absorbance maxima at 246 nm (λ 2). The overlain spectra showed λ max of both drugs and also isoabsorptive points at 238.8 nm (Fig 3.).

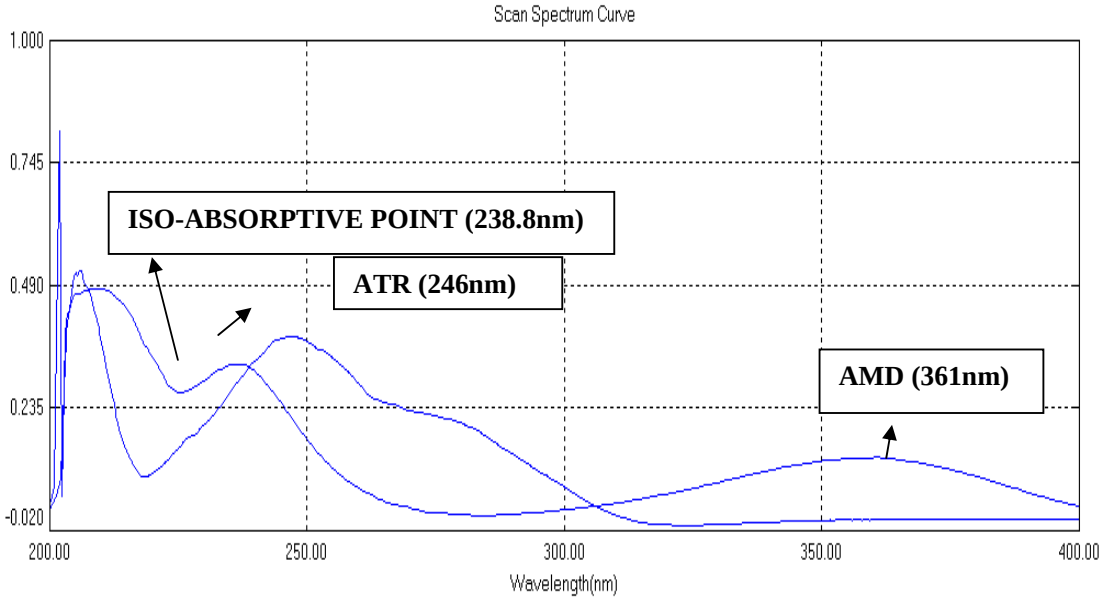

Fig. 3: Overlain spectra of Amlodipine and Atorvastatin

Method I (Simultaneous equation method):

Two wavelengths selected for the method are 361 nm and 246 nm that are absorption maxima of AMD and ATR respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 0.5-30 µg /mL concentrations. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$$Cx = (A_2ay_1 - A_1ay_2) / (ax_2ay_1 - ax_1ay_2)$$

$$Cy = (A_1ax_2 - A_2ax_1) / (ax_2ay_1 - ax_1ay_2)$$

Where, A_1 and A_2 are absorbances of mixture at 361nm and 246 nm respectively, ax_1 and ax_2 are absorptivities of ATR at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of AMD at λ_1 and λ_2 respectively. Cx and Cy are concentrations of ATR and AMD respectively.

Method II (Absorption ratio or Q-Analysis method):

From the overlain spectrum of AMD and ATR, two wavelengths were selected one at 238.8 nm which is the isoabsorptive point for both the drugs and the other at 246 nm which is λ max of ATR. The absorbances of the sample solutions are prepared in a similar manner as in the previous method, were measured and the absorbance ratio values for both the drugs at selected wavelengths were also calculated. The method employs Q-values and the concentrations of drugs in sample solution were determined by using the following formula**,**

Conc. of ATR:

$$C_1 = \frac{Q_m - Q_1}{Q_2 - Q_1} \times \frac{A}{a}$$

Conc. Of AMD:

$$C_2 = \frac{Q_m - Q_2}{Q_1 - Q_2} \times \frac{A}{a}$$

A = Absorbance of sample at isoabsorptive point, a = Absorptivities of AMD and ATR respectively at isoabsorptive point. Q_m, Q_1 and Q_2 are absorbance ratio of mixture, AMD and ATR at Iso-absorptive point to maximum wavelength of one of the component (selected wavelength).

METHOD VALIDATION

Method was validated accordance to ICH guidelines [19-21] for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification and specificity.

Linearity

The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Square method and the drug was linear in the concentration range of 0.5-30 µg/ml for both the drugs. Calibration standards were prepared by spiking required volume of working standard (100µg/mL) solution into different 10 ml volumetric flasks and volume made with methanol to yield concentrations of 0.5, 1, 2, 5, 10, 20 and 30µg/ml. The resultant absorbances of the drugs were measured. Calibration curve was plotted between absorbance of drug against concentration of the drug. These results shown there was an excellent correlation between absorbance and analyte concentration. The linearity graph is presented in [Fig 4.].

Intra-day and Inter-day Precision and Accuracy

Precision and accuracy was studied by quality control samples of standard solutions covering low, medium and high concentrations (3, 15 and 25µg/mL) of linearity range were prepared and measured the absorbance of three replicated samples of each concentration. Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation of the drug at different concentration levels for six injections were less than 2. Precision and accuracy data were shown in [Table 2 and 3.].

Table 1: Optical characteristics

Parameter	238.8nm		246nm		361nm	
	AMD	ATR	AMD	ATR	AMD	ATR
Beer's law limit (µg/mL)	0.5-30	0.5-30	0.5-30	0.5-30	0.5-30	0.5-30
Molar absorptivity	1.30X10⁴	1.78X10⁴	9.44X10³	2.13X10⁴	1.35X10⁴	1 .78X10⁴
Regression equation	**Y=mX+c**					
Slope (m)	0.067		0.061		0.011	
Intercept (c)	0.005		0.003		0.004	
Correlation coefficient (R^2)	0.999		0.998		0.998	
Limit of detection (µg/mL)	0.028		0.054		0.017	
Limit of quantitation (µg/mL)	0.086		0.163		0.052	

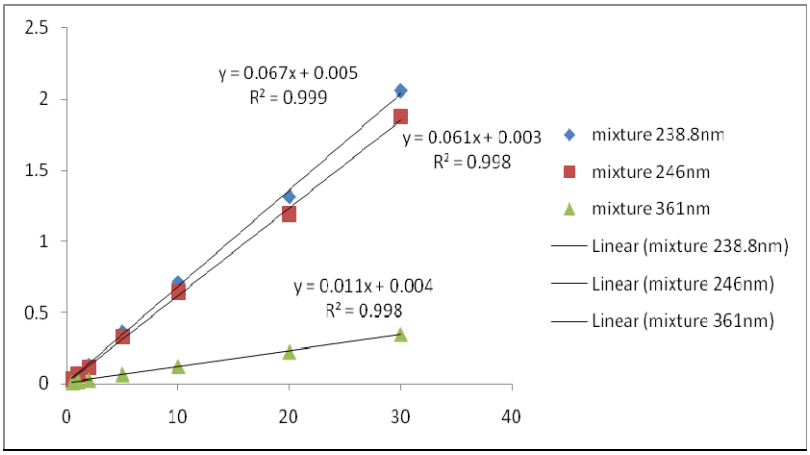

Fig. 4: Linearity graph of Amlodipine and Atorvastatin mixture

Table 2: Intra-day precision and accuracy of Amlodipine and Atorvastatin

	Conc. (µg/mL)		Method-I		Method-II	
			Accuracy	%RSD	Accuracy	%RSD
AMD	3	103.77±1.48	1.429		104.48±0.30	0.295
	15	101.22±0.29	0.295		103.24±0.10	0.103
	25	99.82±0.17	0.172		104.97±0.03	0.037
ATR	3	98.09±0.78	0.797		99.62±0.29	0.295
	15	105.86±0.07	0.073		98.43±0.10	0.103
	25	103.33±0.56	0.548		100.08±0.03	0.035

Values expressed Mean±SD, **(n=6)**

Table 3: Inter-day precision and accuracy of Amlodipine and Atorvastatin

conc. (µg/mL)		Method-I		Method-II	
		Accuracy	%RSD	Accuracy	%RSD
AMD	3	102.88±1.47	1.430	100.64±0.46	0.462
	15	103.12±0.29	1.472	103.24±0.10	0.103
	25	99.82±0.17	0.172	104.97±0.03	0.037
ATR	3	100.08±0.46	0.462	100.64±1.06	1.054
	15	104.42±1.10	1.057	99.31±1.00	1.013
	25	99.80±0.17	0.175	100.24±0.22	0.220

Values expressed Mean±SD, **(n=9)**

Limits of Detection and Quantification

The limit of detection of an analytical method may be defined as the concentration, which gives rise to instrument signal that is significantly different from the blank(signal to noise ratio 3) and LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision (signal to noise ratio 10). The LOD and LOQ was calculated Based on the Standard Deviation of the Response and the Slope. The values were shown in [Table 1.].

APPLICATION OF METHODS TO TABLET DOSAGE FORMS

20 Tablets (Brand Name: ALNAVAS-A, anla bio) were weighed, and an accurately weighed sample of powdered tablets equivalent to 5mg of AMD and 10mg of ATR [equivalent to one tablet]. For analysis of drug, a standard addition method was used. An accurately weighed 5 mg of pure AMD was added to finely powdered samples to bring the ratio of AMD and ATR to 1:1. Quantity of powder equivalent to 10 mg of AMD and 10 mg of ATR was weighed and dissolved in 60 mL of methanol and sonicated for 10 minutes in a 100ml volumetric flask and this solution was filtered through Whatmann No.1 filter paper. The residue was washed with 10ml methanol three times and volume made upto 100ml with methanol. The solution obtained was diluted with the Methanol so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates. In Method I, the concentration of both AMD and ATR were determined by measuring the absorbance of the sample at 246 nm and 361nm. For Method II, the concentration of both AMD and ATR were determined by measuring absorbance of the sample at 238.8 nm and 246 nm and values were substituted in the respective formula to obtain concentrations. Results of tablet analysis are shown in [Table 4.].

RESULTS AND DISCUSSION

Drug content in tablet (amount found) was directly found from equations for both the methods. Standard deviations and Coefficient of variation was calculated (Table 2and 3). The low standard deviation values indicated repeatability, accuracy and reproducibility of the methods. Reproducibility, reliability and interference was also confirmed by recovery studies. Thus, it can be concluded that the methods developed were simple, accurate, sensitive and precise. Statistical analysis and drug recovery data showed that both methods are sensitive, accurate and precise. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully in simultaneous estimation of Atorvastatin calcium and Amlodipine besylate in marketed formulations.

Table 4: Recovery study from formulation of Amlodipine and Atorvastatin

Labeled amount		Method-I			Method-II	
		(Alnavas-A)	Accuracy	% RSD	Accuracy	%RSD
AMD	5mg	101.22±0.69	0.694		103.24±0.10	0.203
ATR	10mg	99.86±0.06	0.063		98.43±0.10	0.503

Values expressed Mean±SD, **(n=5)**

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