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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ATORVASTATIN CALCIUM AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORMS

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ABSTRACT: A simple and economical dual wavelength spectrophotometric method has been developed for the simultaneous estimation of atorvastatin calcium and amlodipine besylate in their combined dosage forms. The method was based on property of additivity of absorbances. The two wavelengths on amlodipine besylate curve were found out where it showed same absorbance, which were 257.4 and 360.0 nm. At 360.0 nm, amlodipine besylate showed some absorbance while atorvastatin calcium showed zero absorbance. Both the drugs gave absorbance at 257.4 nm. The method involved solving of an equation based on measurement of absorbances at two wavelengths 257.4 and 360.0 nm. The proposed method was found to be simple, economical, accurate and reproducible for the routine analysis of both drugs in tablet dosage forms.
Key words – Spectrophotometric, atorvastatin calcium, amlodipine besylate.

INTRODUCTION

Atorvastatin calcium[1] is a synthetic lipid lowering agent which inhibits HMG–CoA reductase and amlodipine besylate [2] is a calcium antagonist drug effective in hypertension and angina pectoris. The combination drug product of atorvastatin calcium (ATV) and amlodipine besylate (AML) has been introduced in the market; co-administration of AML with ATV demonstrated statistically significant dose-related reductions in systolic blood pressure (SBP), diastolic blood pressure (DBP) and LDL-C in patients with co-morbid hypertension and dyslipidemia[3]. Chemically ATV is [R-(R*, R*)]-2-(4-fluorophenyl)-ß, dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate[4] and AML is 2-[(2-Amino ethoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester[5].

HPLC methods are official in IP[6] for the estimation of ATV while in IP[7], BP[8], EP[9] and USP[10] for the determination of AML, but they do not involve simultaneous determination of ATV and AML. Detailed survey of literature for ATV revealed several methods based on different techniques, viz. HPLC[11,-13] and LC-MS[14-16] for its determination in plasma/serum; HPLC[17] for its determination in human serum and pharmaceutical formulations; HPLC[18,19]; HPTLC[20] for its determination in pharmaceuticals. Similarly, survey of literature for AML revealed methods based on spectrophotometry[21], RP-HPLC[22] using fluorescence detection, HPLC-tandem mass spectrometry[23,24], RP-HPLC using UV detection[25,26], HPLC[27-31] in combination with other drugs, Flow injection analysis using UV-detection[32], HPTLC[33], stability indicating

HPLC[34] and stability indicating HPLC[35] in combination with benazepril hydrochloride have been reported. Spectrophotometric[36], HPLC[37,38] and HPTLC[39] methods have been reported for simultaneous determination of ATV and AML. The reported HPLC[37,38] methods involves costly sophisticated instrumentation and time consuming process while HPTLC[39] method has no reproducibility. Spectrophotometric[36] method for simultaneous determination of ATV and AML involves selection of four different wavelengths which is tedious and time consuming as well as was not applied for marketed dosage forms. In the present investigation an attempt has been made to develop a simple, economic, accurate, reproducible and less time consuming spectrophotometric method for the simultaneous estimation of ATV and AML in their combined dosage forms at only two different wavelengths. The method was based on dual wavelength data processing program (dual wavelength spectrophotometry or DW spectrophotometry). The proposed method was successfully applied for simultaneous determination of ATV and AML in combined dosage forms that are available in market.

MATERIAL AND METHODS

A double beam UV-visible Spectrophotometer (Shimadzu model UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was employed. Kindly gifted reference standards of Atorvastatin calcium and amlodipine besylate (Torrent Research Center, Gandhinagar) with purity of 98.30% and 99.77%, respectively and were used without further purification for the study. Methanol (A.R. Grade, S.D. Fine Chem. Pvt. Ltd., Mumbai) was used.

Preparation of standard and sample solutions:

Accurately weighed 25 mg of ATV and AML standard powder was transferred in two separate 25 ml volumetric flasks, were dissolved in methanol and volumes were made up to mark with same solvent. From the above solutions of ATV and AML, 10 mL aliquots were pipetted and transferred in two separate 100 ml volumetric flasks, diluted up to mark with methanol to obtain final solutions of 100 µg/ml.

Twenty tablets (each tablet contains 10 mg atorvastatin and 5 mg amlodipine) were accurately weighed, their mean weight was determined, and were ground to fine powder in a glass mortar. An amount of powdered mass equivalent to 25 mg of ATV and 12.5 mg of AML was weighed and transferred in conical flask. The drugs from powder were dissolved and extracted with methanol. To ensure complete extraction of drugs it was sonicated for 30 min. The extract was filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extract and washing were pooled and transferred to a 25 ml volumetric flask and volume was made with methanol. Five ml aliquot from above solution was transferred in 50 ml volumetric flask and volume was adjusted with methanol up to mark. This solution was expected to contain 100 µg/ml of ATV and 50 µg/ml of AML. From this solution, 7.5 ml aliquot was diluted to 25 ml with methanol to achieve final concentration of ATV (30 µg/ml) and AML (15 µg/ml).

Selection of wavelength for estimation of ATV and AML:

Absorbance spectrum of pure AML was scanned in the spectrum basic mode. Using the cursor function, the absorbance corresponding to 360.0 nm (wavelength λ_1, the wavelength of reasonable absorbance for AML) was noted from the spectrum. Then the cursor function was moved along with peak curve until the absorbance equal to that of absorbance at 360.0 nm was found. The wavelength obtained corresponding to this absorbance value was 257.4 nm ((λ_2). Absorbance spectrum of pure ATV was also scanned in spectrum basic mode. ATV showed some absorbance value at 257.4 nm ((λ_2) while it does not show any absorbance at 360.0 nm ((λ_1). The absorbance value at 360.0 nm was due to AML only in the combined mixture of both drugs and was selected for the measurement of AML. The absorbance of various dilutions of ATV and AML in methanol was measured at λ_1 and λ_2. At these two wavelengths, absorbance difference for AML at any concentration level was found to be zero while for ATV, absorbance difference was found to increase concomitantly as concentration was increases.

Calibration curve for ATV and AML:

Appropriate aliquots from the stock solution of ATV and AML were used to prepare three different sets of dilutions, Series A, B and C as follows. Series A and B consisted of different concentrations of ATV (5-30 µg/ml) and AML (10-60 µg/ml), respectively. Aliquot of the stock solutions of ATV and AML (100 µg/ml of each) was pippeted out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 5-30 µg/ml and 10-60 µg/ml for ATV and AML, respectively. Series C comprised of mixture of ATV and AML having varying concentrations of ATV (5-30 µg/ml) and AML (10-60 µg/ml). The solutions were prepared by pipetting out 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml of the stock solution of ATV (100 µg/ml) and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 ml of the stock solution of ATV (100 µg/ml), respectively in to a series of 10 ml volumetric flasks and the volume was made up to mark with methanol.

Analysis of combined tablet dosage form:

The absorbance of final sample solution was measured against methanol as blank at 257.4 nm and 360.0 nm.

The amount of ATV and AML was computed using respective equation of straight line.

RESULTS AND DISCUSSION

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths were chosen: (i) First wavelength, λ_1 at which zero absorbance of ATV and reasonable absorbance of AML was observed. (ii) Second wavelength, λ_2 was the wavelength at which the absorbance of the AML was equal to the absorbance at λ_1[40,41].

In the proposed procedure the absorbance of ATV alone in mixture of ATV and AML was determined using dual wavelength data processing program. To remove interference of AML to the absorbance at 257.4 nm (λ_2), the wavelength of reasonable absorbance for ATV, another wavelength 360.0 nm (λ1) was found out at which the absorbance of ATV was zero. This was confirmed by various dilutions of AML in methanol at 257.4 nm and 360 nm, respectively. The absorbance at these two selected wavelengths was found to be equal. These two wavelengths were employed to determine the concentration of ATV from the mixture of ATV and AML. The difference in absorbance at these two wavelengths ($A_{257.4}$-$A_{360.0}$) cancels out the contribution of absorbance of AML in measurement of ATV at 257.4 nm and the difference in absorbance was proportional to the concentration of ATV in the mixture. It was found that this difference in absorbance values was linear in the range of 5-30 µg/ml of ATV with correlation coefficient 0.9991 (Table 1).

Further, the absorbance value at 360.0 nm was only due to AML, as ATV has zero absorbance at this wavelength. The absorbance values were found to be linear over the range of 10-60 µg/ml of AML with correlation coefficient of 0.9984 (Table 2). These results confirm the suitability of the proposed method for the simultaneous determination of ATV and AML from their mixture.

Regression analysis (Table 3) for series A and C shows no difference in the equations of straight line and thus indicates that there is no interference of AML in determination of ATV. Same way for series B and C, no difference in the equations of straight line indicates that there is no interference of ATV on measurement of AML. From the series C, the limit of detection (LOD) and limit of quantification (LOQ) were determined by visual methods as suggested in ICH guidelines, which were found to be 1 µg/ml and 5 µg/ml, respectively for ATV and 3 µg/ml and 10 µg/ml, respectively for AML. Sensitivity parameters such as molar absorptivity (L/mole/cm) and Sandell's sensitivity (µg/ml/cm^2 /0.001 Absorbance units) were found to be 4.24×10^4 and 2.16×10^{-2}, respectively for ATV and 6.12×10^3 and 8.54×10^{-2}, respectively for AML.

Accuracy was checked by recovery study at 3 different concentration levels, i.e., a multilevel recovery study. The tablet samples were spiked with an extra 50, 100, 150 % of standard ATV and AML, and the mixtures were analyzed by proposed method. Results of the recovery study are shown in Table 4 suggested that method was accurate for the simultaneous estimation of ATV and AML from their combination drug products. The method was applied for the analysis of three marketed formulations containing ATV 10 mg and AML 5 mg per tablet. The results of analysis of tablet formulations are shown in Table 5. All of them meet pharmacopoeial requirement of ATV and AML.

CONCLUSIONS

The proposed method is based on dual wavelength data processing and only requires measurement of absorbance at selected wavelengths. The values of percentage coefficient of variance (CV, %) were 1.72 and 1.59 for determination of ATV and AML, respectively, showing reproducibility of the method. Interference studies revealed that the common excipients and other additives usually present in the tablet dosage forms did not interfere in the proposed method for estimation of both drugs. The proposed method was found to be simple, rapid, economical, accurate and precise. It can be useful for routine in-process quality control and simultaneous estimation of ATV and AML from their combined tablet dosage forms.

Table 1: Determination of ATV alone and in presence of AML by proposed DW Spectrophotometry

Series A				Series C			
Composition of mixture (µg/ml)		Absorbance at 257.4 nm ± S.D. (n=5)	CV, %	Composition of mixture (µg/ml)		Absorbance at 257.4 nm – Absorbance at 360 nm ± S.D. (n=5)	CV, %
ATV	AML			ATV	AML		
5	0	0.313 ± 0.007	2.41	5	10	0.314 ± 0.007	2.26
10	0	0.494 ± 0.010	1.99	10	20	0.495 ± 0.008	1.66
15	0	0.667 ± 0.013	2.00	15	30	0.666 ± 0.011	1.73
20	0	0.872 ± 0.016	1.79	20	40	0.874 ± 0.013	1.54
25	0	1.055 ± 0.016	1.56	25	50	1.057 ± 0.015	1.44
30	0	1.220 ± 0.006	1.86	30	60	1.218 ± 0.020	1.68

n= Number of determinations.

Table 2: Determination of AML alone and in presence of ATV by proposed DW Spectrophotometry

Series B				Series C			
Composition of mixture (µg/ml)		Absorbance at 360 nm ± S.D. (n=5)	CV, %	Composition of mixture (µg/ml)		Absorbance at 360 nm ± S.D. (n=5)	CV, %
ATV	AML			ATV	AML		
0	10	0.134 ± 0.002	1.76	5	10	0.138 ± 0.002	1.81
0	20	0.223 ± 0.003	1.54	10	20	0.224 ± 0.004	1.76
0	30	0.352 ± 0.005	1.57	15	30	0.351 ± 0.006	1.59
0	40	0.446 ± 0.006	1.41	20	40	0.448 ± 0.007	1.53
0	50	0.560 ± 0.010	1.77	25	50	0.565 ± 0.008	1.38
0	60	0.671 ± 0.011	1.65	30	60	0.670 ± 0.010	1.47

n= Number of determinations.

Table 3: Regression analysis data of the calibration curve obtained using series A, B and C

Series	Composition of the sample solution		Regression equation of the curve	Correlation coefficient
	ATV (µg/ml)	AML (µg/ml)		
A	5-30	0	Y = 0.0367X + 0.1279	0.9993
B	0	10-60	Y = 0.0108X + 0.0187	0.9985
C	5-30	10-60	*Y = 0.0367X + 0.1293	0.9991
			**Y = 0.0108X + 0.0213	0.9984

Y is absorbance and X is concentration in µg/ml.
*Regression equation for ATV,
** Regression equation for AML

Table 4: Recovery study of ATV and AML from tablet formulations (n=3)

Label Claim (mg/tablet)		Amount of standard Added(%)		Total Amount of standard Added(mg)		Amount of standard Recovered(mg)		% Recovery* ± SD	
ATV	AML	ATV	AML	ATV	AML	ATV	AML	ATV	AML
10	5	50	50	5.00	2.50	5.06	2.44	101.3 ± 1.2	97.5 ± 1.3
10	5	100	100	10.00	5.00	10.21	4.90	102.1 ± 1.8	98.1 ± 1.6
10	5	150	150	15.00	7.50	14.88	7.33	99.2 ± 1.4	97.8 ± 1.8

* Indicates that each value is mean ± standard deviation of three determinations

Table 5: Analysis of pharmaceutical formulations

Formulation	ATV % found ± S.D. (n=5)	AML % found ± S.D. (n=5)
Tablet-1	98.6 ± 1.17	96.8 ± 1.63
Tablet-2	97.5 ± 1.66	97.4 ± 0.79
Tablet-3	99.1 ± 0.82	97.7 ± 1.58

n= Number of determinations.

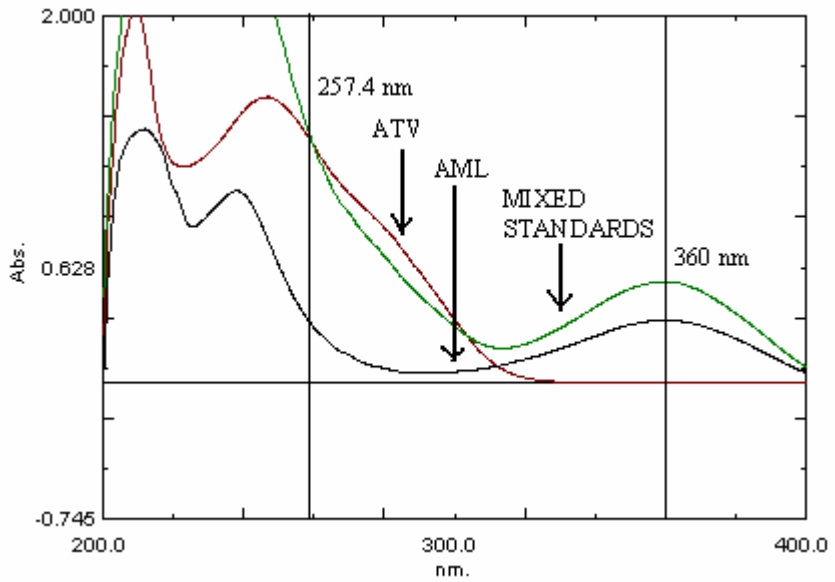

Figure 1: Overlain spectra of the drugs

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