DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF ATORVASTATIN CALCIUM AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORM

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ABSTRACT

Atorvastatin calcium (AT) is hypolipidemic agent and Amlodipine besylate (AM) is calcium channel blocker. The combination of both drugs is used in treatment of hypertension. The present communication deals with development of simple, sensitive, rapid and economical method for simultaneous estimation of atorvastatin calcium and amlodipine besylate in combined dosage form. The AT and AM stock solutions are prepared in (50:50 V/V) methanol: water. The method of analysis is derivative spectroscopy to eliminate spectral interference by measuring absorbances at 241nm and 250 nm for AM and AT respectively. The AT and AM are linear in concentration range of 0-14 µg/ml and 0-7 µg/ml respectively. The limit of detection (LOD) and limit of quantitation (LOQ) of AM was 0.29 and 0.75 µg respectively. The limit of detection (LOD) and limit of quantitation (LOQ) of AT was 0.21 and 0.60 µg respectively. The results of analysis were validated by ICH Q2B (R1). The results of recovery studies and precision were found to be within limits.

Keywords: UV Spectrophotometry, Amlodipine, Atorvastatin, Derivative Spectrophotometry

INTRODUCTION

Atorvastatin calcium (AT)[(ßR,dS)-2-(4-fluorophenyl)- β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino) carbonyl]-1H-pyrrole-1-heptenoic acid calcium salt is a hypolipidemic agent, which inhibits HMG CO-A reductase, enzyme involved in lipid synthesis[1].

Amlodipine besylate (AM), [3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3, 5-dicarboxylate benzenesulphonate is a calcium channel blocker, used in hypertension treatment. Both are official in BP and EP[2-3].

Several spectrophotometric and chromatographic methods methods are reported for estimation of AT single drug or in combination with other drugs[4-7]. A number of spectrophotometric and chromatographic methods are reported to estimate AM as single drug or in combination with other drugs[8-13]. For simultaneous estimation of AT and AM, HPLC, simultaneous equation, dual wavelength method are reported[14-18]. But derivative spectrophotometric method is not reported which is simple, precise and accurate.

MATERIALS AND METHODS

Chemicals: AT and AM were procured from Hetero Drugs Limited, (H.P.) as gift sample. Methanol AR grade (Merck Pvt. Ltd.) was used for analysis. The distilled water was prepared using glass distillation assembly in laboratory.

Instrument: Spectrophotometric analysis was carried out on JASCO model V- 530 UV-Visible double beam high speed scanning spectrophotometer with a single monochromator with a 1200 grooves/mm concave grating. Detector was silicon photodiode (S1337). Light source used were deuterium lamp (190 to 350 nm) and halogen lamp (330 to 1100 nm).The instrument is having wavelength accuracy of ± 0.3 nm and baseline stability of ± 0.001 Abs. / Hrs.

Preparation of standard stock solutions

Standard stock solutions of AT and AM were prepared by dissolving 10 mg each in 25 ml of (50:50 V/V) methanol:water solvent mixture separately in 100 ml volumetric flask and finally making up the volume up to the mark with same solvent system. The concentration of stock solutions of both drugs is 1000 µg/ml. The required solutions are prepared by diluting these solutions.

Selection of wavelength for analysis

These standard stock solutions were further diluted to get final dilutions containing 10 µg/ml of AT and AM each[10]. These dilutions were scanned in UV region (400-200nm). The obtained individual spectra were overlain (Fig 1). This overlain zero order spectrum was then converted to first order derivative spectrum (Fig 2). From derivative spectrum, wavelength of 241nm and 250 nm for AT and AM were selected as estimation wavelengths, respectively to avoid interference of each other at respective wavelengths[19].

Linearity study of AT and AM

Using standard stock solutions of both drugs, seven mixed standard solutions were prepared containing 0µg/ml and 7 µg/ml, 2 µg/ml and 6 µg/ml, 5 µg/ml and 4 µg/ml, 4 µg/ml and 6 µg/ml, 3 µg/ml and 8 µg/ml, 2 µg/ml and 10 µg/ml, 1 µg/ml and 12 µg/ml, 0 µg/ml and 14 µg/ml of AT and AM respectively[20]. All mixed standard solutions were scanned over 400-200nm and derivatised to measure substantial absorbance at 241 for AT and at 250 for AM respectively. Calibration curves for both drugs were plotted using absorbance values and concentrations of each drug in the mixed standard solutions. It was found that atorvastatin calcium is linear in concentration range of 0- 14 µg/ml with correlation coefficient of 0.9994 while that of amlodipine besylate is linear in concentration range of 0- 7 µg/ml with correlation coefficient of 0.9992.

Analysis of marketed formulation

The commercial formulation ZIVAST AM (Hetero Drugs Limited) was purchased from local pharmacy. The average weight of each tablet (before and after removing coat) was calculated using 20 tablets. The twenty tablets were powdered finely in glass mortar after removing coatings. The powdered sample equivalent to 10mg of AT (5 mg AM) was taken in 30 ml methanol: water solvent mixture, shaken well to dissolve drugs and transferred quantitatively to 100 ml volumetric flask after filtering through Whatman filter paper twice. Finally volume was made up. Three solutions were prepared from this stock solution of tablet covering entire calibration range. These solutions were further diluted so that final dilutions will lie in workable limit of 0-14µg/ml for AT and 0-7 µg/ml for AM. The absorbance values of both drugs were recorded at respective wavelength present in derivatised spectra of drugs. The concentrations of both drugs were calculated using calibration curve data. The results of tablet analysis are reported in Table 1.

RESULT AND DISCUSSION

The combination of AT and AM is used in treatment of hypertension. Hence it is necessary to develop method for simultaneous estimation of AT and AM. Previously reported spectrophotometric methods were found to be less sensitive and selective for estimation of both drugs.

In present communication we have succeeded in development and validation of simple, accurate, precise, sensitive and selective derivative spectrophotometric method for simultaneous estimation of both drugs form tablet formulations.

Validation

The method was validated according to ICH Q2B (R1) guidelines, [21] meant for validation of analytical methods to check accuracy, precision, linearity range, limit of detection, limit of quantification and specificity.

Accuracy: Recovery studies were performed by standard addition method at three levels i.e., 80%, 100% and 120%. The known amounts of standard AT and AM were added to pre-analyzed laboratory samples and they were subjected to analysis by the proposed method. Results of recovery studies are shown in Table 1. The results of recovery studies were found to be in range of 98.43 to 101.47 with standard deviation value of 1.765.

Precision: Precision study was performed to find out intra-day and inter-day variations. The results of precision studies are reported in Table 2 and values of standard deviation less than 2% indicates high degree of precision.

Limit of detection (LOD) and limit of quantitation (LOQ); The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y-intercepts and slope of the regression lines were used for calculation. The AT and AM are linear in concentration range of 0-14 µg/ml and 0-7 µg/ml respectively.

The LOD and LOQ of AM was 0.29 and 0.75 µg respectively.

The LOD and LOQ of AT was 0.21 and 0.60 µg respectively.

Table 1: Result of Tablet Analysis and Recovery Studies

Drug	Label Claim (mg/ tablet)	% Amount Found[a] ± R.S.D.[b]	% Recovery[a] ± R.S.D.[b]
AT	10	100.06 ± 0.8859	99.72 ± 0.5074
		99.59 ± 0.5019	99.45 ± 0.5277
		99.72 ± 0.5074	100.04 ± 0.3240
AM	5	99.21 ± 1.0107	99.95±0.3559
		99.59 ± 0.5019	99.59±0.7455
		98.37 ± 0.7846	100.45 ± 0.8539

a: Average of Three Determinations,   b: Relative Standard Deviation

Table 2: Result of Precision Studies

% Label claim Estimated[a] (Mean ±R.S.D.[b])	Day 1		Day 2	
	Morning	Evening	Morning	Evening
AT	99.98±0.3854	100.04 ± 1.2422	98.11 ±0.6098	99.98±0.3854
AM	101.02±0.9637	99.40 ± 1.2844	98.98 ± 1.342	101.11±0.6739

a: Average of Nine Determinations,   b: Relative Standard Deviation
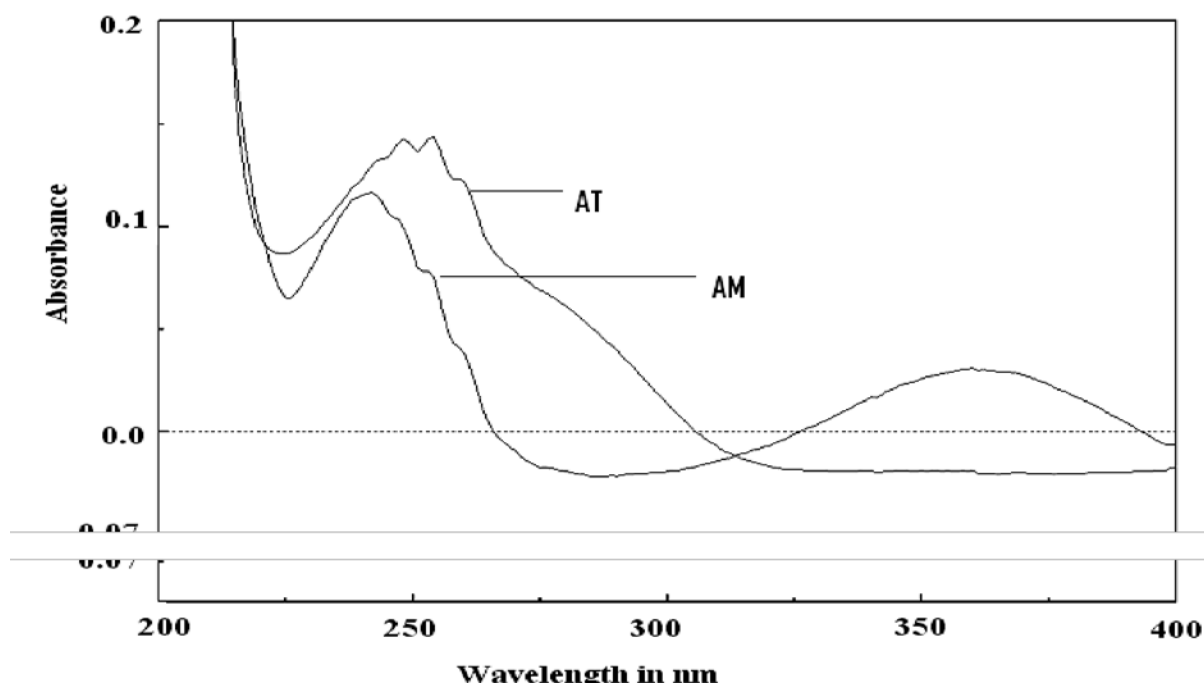

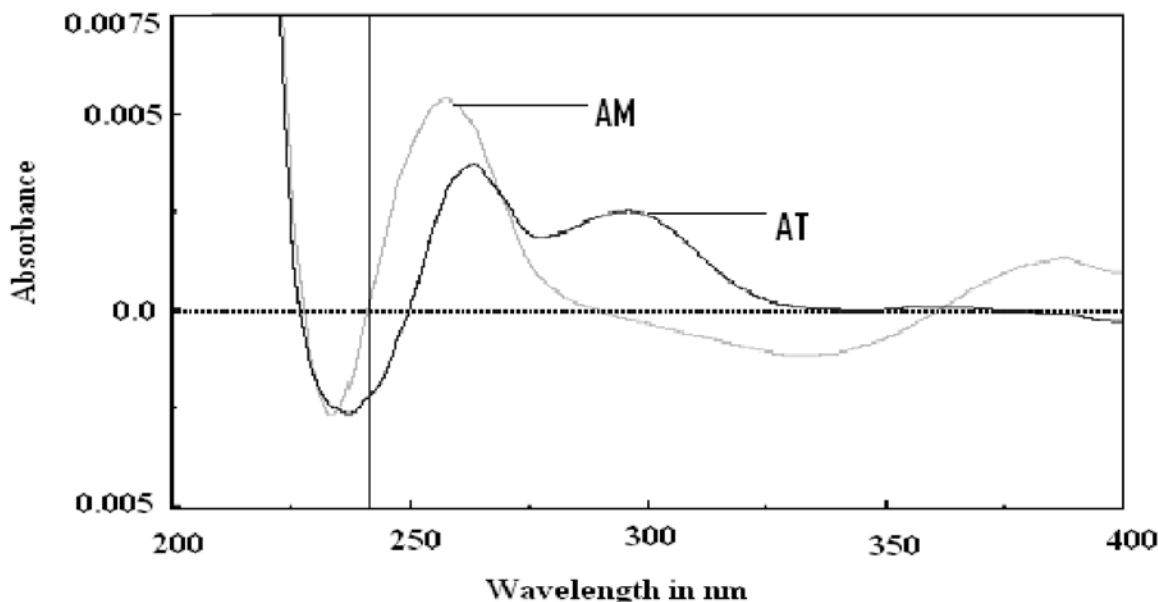Fig. 1: Overlain Spectra of AT and AM in Zero Order Derivative Mode

Fig. 2: Overlain Spectra of AT and AM in First Order Derivative Mode

CONCLUSION

The proposed derivative spectrophotometric method is found to be accurate, precise, economic and rapid for simultaneous estimation of AT and AM. It satisfactorily eliminates interference from excipients. Hence it can be employed for routine analysis of both drugs in marketed formulations in quality control laboratories.

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