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 241 nm 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

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

SMITA T. KUMBHAR, SWAPNIL D. JADHAV, NEELA M. BHATIA AND MANISH S. BHATIA*

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Kolhapur-416013(M.S.) India.
Email: bhatiamanish13@gmail.com

Received: 9 May 2011, Revised and Accepted: 21 June 2011

INTRODUCTION

Atorvastatin calcium (AT) [(8R,8S)-2-((4-fluorophenyl)-3,8-dihydroxy-5-[(1-methyl-1H-pyrrole-1-yl)carbonyl]-3-phenyl-4-[((phenylamino)methyl]-1H-pyrrole-1-heptenoic acid calcium salt is a hypolipidemic agent, which inhibits HMG CO-A reductase, enzyme involved in lipid synthesis1.

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 drugs8-13. For simultaneous estimation of AT and AM, HPLC, UV Spectrophotometry, Amlodipine, Atorvastatin, Derivative Spectrophotometry

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 each19. These dilutions were scanned in UV region (400-200 nm). 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 241 nm and 250 nm for AT and AM were selected as estimation wavelengths, respectively to avoid interference of each other at respective wavelengths17.

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 respectively20. All mixed standard solutions were scanned over 400-200 nm 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 0-14 µg/ml and 0-7 µg/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, which 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>
<thead>
<tr>
<th>Drug</th>
<th>Label Claim (mg/tablet)</th>
<th>% Amount Found ± R.S.D.</th>
<th>% Recovery ± R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>10</td>
<td>100.06 ± 0.8859</td>
<td>99.72 ± 0.5074</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.59 ± 0.5019</td>
<td>99.45 ± 0.5277</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.72 ± 0.5074</td>
<td>100.04 ± 0.3240</td>
</tr>
<tr>
<td>AM</td>
<td>5</td>
<td>99.21 ± 1.0107</td>
<td>99.95 ± 0.3559</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.59 ± 0.5019</td>
<td>99.59 ± 0.7455</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98.37 ± 0.7846</td>
<td>100.45 ± 0.8539</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Label claim Estimated ± R.S.D.</th>
<th>Day 1 Morning</th>
<th>Day 1 Evening</th>
<th>Day 2 Morning</th>
<th>Day 2 Evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>99.98 ± 0.3854</td>
<td>100.04 ± 1.2422</td>
<td>98.11 ± 0.6098</td>
<td>99.98 ± 0.3854</td>
</tr>
<tr>
<td>AM</td>
<td>101.02 ± 0.9637</td>
<td>99.40 ± 1.2844</td>
<td>98.98 ± 1.342</td>
<td>101.11 ± 0.6739</td>
</tr>
</tbody>
</table>

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

---

**Fig. 1: Overlain Spectra of AT and AM in Zero 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.

ACKNOWLEDGEMENT
The authors are thankful Principal, Bharati Vidyapeeth College of Pharmacy, Kolhapur for providing facilities and to Hetero Drugs Limited, Himachal Pradesh for providing gift sample of drugs AT and AM for this research project.

REFERENCES