Int. J. Chem. Sci.: 8(1), 2010, 701-710

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ATORVASTATIN CALCIUM AND AMLODIPINE BESYLATE IN COMBINED TABLET DOSAGE FORM BY AREA UNDER CURVE METHOD

DHARMESH J. JANI, MANZOOR AHMED*, SATISHKUMAR A. SHETTY, B. K. SRIDHAR and JIGNESH S. SHAH

Department of Pharmaceutical analysis, National College of Pharmacy,
SHIMOGA – 577201 (K.S.) INDIA

ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid UV spectrophotometric method with subsequent validation using ICH suggested approach for the determination of atorvastatin calcium (ATR) and amlodipine besylate (AML) using methanol as the solvent. The proposed area under curve method involves the measurement of area at selected analytical wavelength ranges and performing the analysis using "Cramer's Rule" and "Matrix Method". Two analytical wavelength ranges selected were 256-238.5 nm and 368-352 nm for the estimation of ATR and AML. The linearity of the proposed method was investigated in the range of 5-50 μg/mL (r = 0.9998) for ATR and 5-50 μg/mL (r = 0.9997) for AML, resptectively. The percentage mean recovery was found to be 99.83% for ATR and 99.60% for AML. Also the method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD value indicating high grade of precision of the method.

Key words: UV spectrophotometric estimation, Atorvastatin calcium, Amlodipine besylate, Validation.

INTRODUCTION

Atorvastatin calcium (ATR) is chemically described as [R-(R*, R*)]-2-(4-Fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl] -1H-pyrrole-1-heptanoic acid: calcium salt (2 : 1) trihydrate[1,2]. ATR is lever selective competitive inhibitor of 3-hydroxy-3-methylglutaryl Co-enzyme A (HMG Co A) reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl Co enzyme A to mevalonate, a

*Author for correspondence; Ph.: +919448204746, +918182279861; Fax : +918182273796;
E-mail: ms_manzoor@yahoo.com

precursor of cholesterol biosynthesis. It also lowers elevated total and LDL cholesterol, apolipoprotein-B, and triglyceride levels in patients with primary hypercholesterolemia and mixed dislipidemia[3-5]. ATR is rapidly absorbed after oral administration; however, due to presystemic clearance in gastro intestinal mucosa and metabolism in liver, its absolute bioavalability is approximately 12% and low plasma concentration is achieved following administration of the drug[6-8].

Amlodipine besylate (AML) is chemically described as (R, S) 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester benzene sulphonate[1,2]. AML is a dihydropyridine derivative with calcium antagonist activity. AML like other calcium channel blockers inhibits the slow channel influx of calcium into cardiac and vascular tissues. AML has peripheral vasodilatory action and also produces vasodilation in coronary vascular beds. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina[3-5].

Survey of literature reveals that only few methods have been developed for the determination of ATR and AML individually and in combination with other drugs. Stability indicating RP-HPLC methods have also been developed for the determination of both the drugs individually[9-16]. Hence, an attempt has been made to develop a simple, accurate, precise and reproducible area under curve method for simultaneous estimation of ATR and AML in combined dosage form with validation as per recommendation of ICH guidelines[17].

EXPERIMENTAL

Chemicals and reagents

The working standard samples of ATR and AML were gifted from Torrent Pharmaceuticals Ltd. Ahmedabad, India. The tablet formulation of ATR and AML (Label claim: Atorvastatin 10 mg, as Atorvastatin calcium and Amlodipine 5 mg, as Amlodipine besylate), Storvas tablets (Ranbaxy Laboratories Ltd. Asalali, Ahmedabad) were purchased from the local market. Acetonitrile, ammonium acetate (HPLC grade), glacial acetic acid (AR grade) and glass double distilled water obtained from E. Merck Ltd.

Instrumed used

A Shimadzu UV/visible spectrophotometer (Model 1700) with 1 cm matched quartz cells was used for spectrophotometric analysis. The spectra were recorded using specific program of the instrument (UV Probe 2.1), having specifications as, spectral band width 2 nm, wavelength accuracy ± 0.5 nm and wavelength readability 0.1 nm increment.

Preparation of standard stock solution and selection of analytical wavelength

The standard stock solutions 100 μg/mL each of ATR and AML were prepared separately by dissolving accurately weighed working standards in small proportions of methanol and later diluted to desired volume with the same.

Appropriate dilutions of the above stock solutions were done to obtain a working solution of 30 μg/mL each of ATR and AML. Both the solutions were separately scanned in the wavelength region of 400-200 nm in the "Spectrum mode". On examination of the spectra, 256-238.5 nm was selected as working wavelength range for ATR and 368-352 nm was selected as working wavelength for AML, as at the above selected wavelength range the area under curve (AUC) remains constant, ideally obeying "Cramer's Rule" and Matrix Method" (Fig. 1 and 2).

Prepatations of calibration curves

Appropriate dilutions were prepared form the standard stock solutions of ATR and AML, respectively to obtain a working concentration range of 5-50 μg/mL for both the drugs. Area under curve of the above solutions of ATR and AML were measured at their respective selected analytical wavelength ranges and their calibration curves were prepared by plotting area under curve (AUC) against concentration (Fig. 3 and 4).

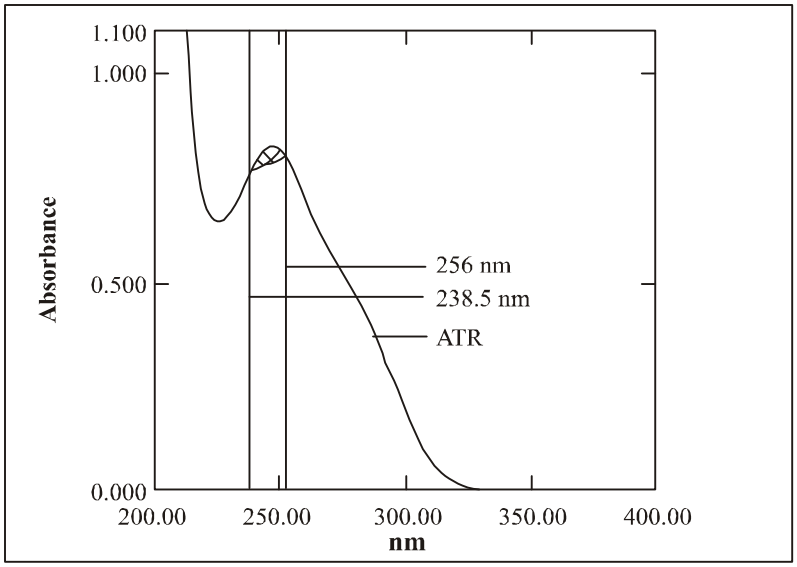

Fig. 1: Spectra showing area under curve of atorvastatin calcium at 368 – 352 nm and 256 – 238.5 nm, respectively

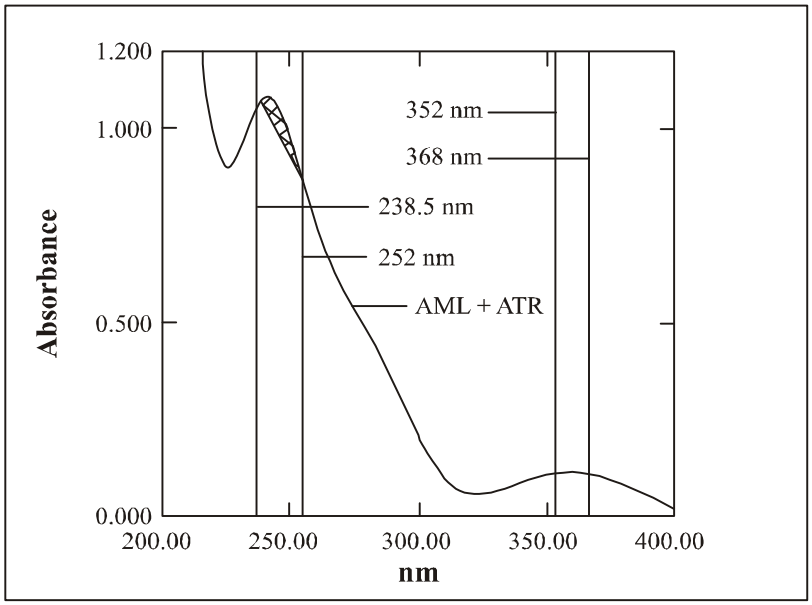

Fig. 2: Spectra showing area under curve of amlodipine besylate at 368 – 352 nm and 256 – 238.5 nm, respectively

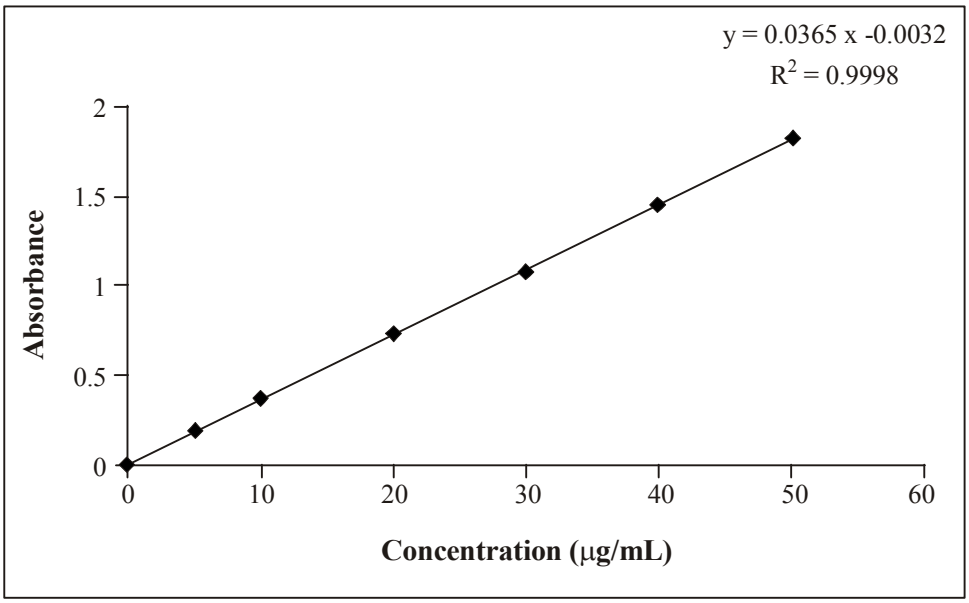

Fig. 4: Calibration curve of atorvastatin calcium at 256-238.5 nm in methanol by area under curve method

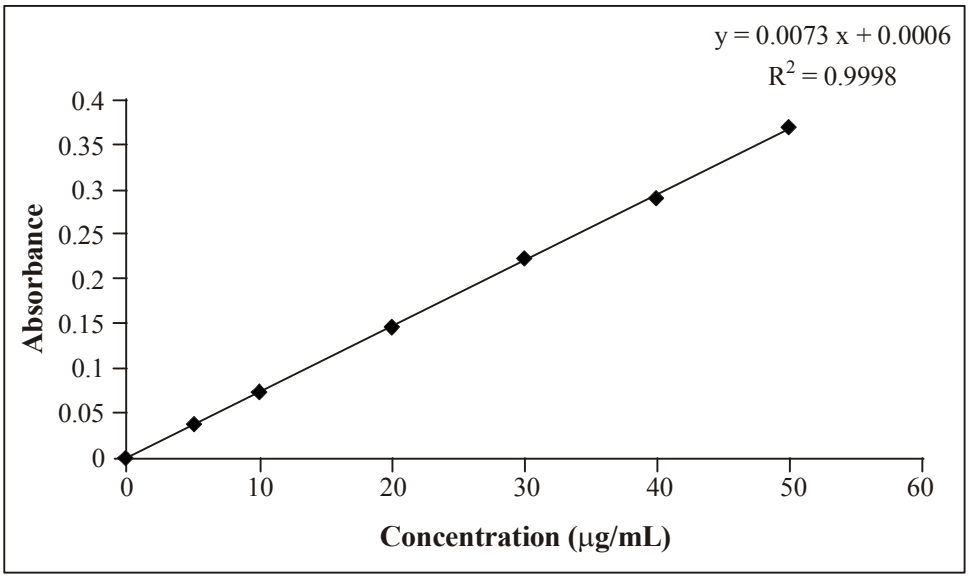

Fig. 4: Calibration curve of atorvastatin calcium at 256-238.5 nm in methanol by area under curve method

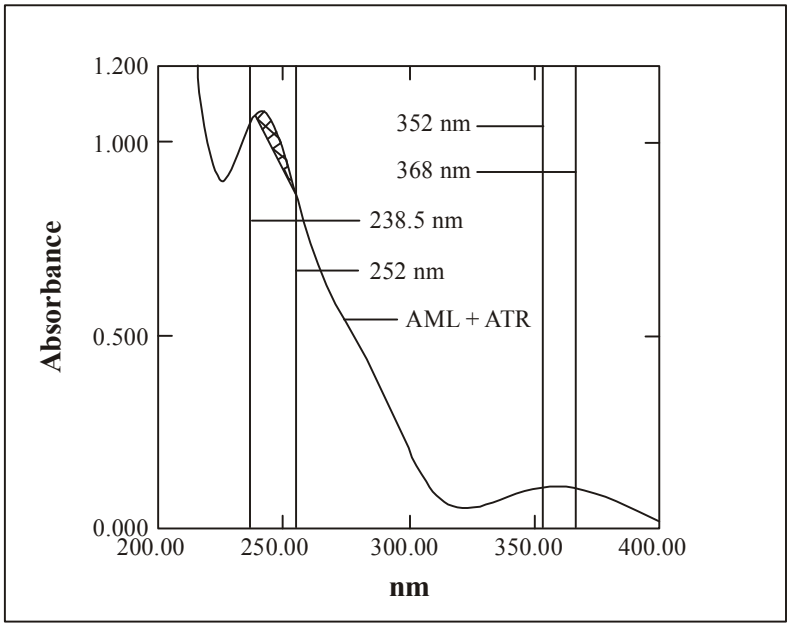

Fig. 5: Spectra showing area under curve of mixture at 368 – 352 nm and 256 – 238.5 nm, respectively

Analysis of tablet dosage form

Twenty tablets were weighed, their mean weight was determined and finally they were crushed to obtain a fine powder. An amount of powdered mass equivalent to one tablet content was transferred into a 100 mL volumetric flask and dissolved in sufficient quantity of methanol. The contents were ultrasonicated for 20 minutes and the final volume was made up to the mark with methanol. The prepared solution was then filtered through Whatmann filter paper No. 41. Appropriate aliquot was pippetted out from the standard stock solution and was further diluted to obtain a mixture containing 20 μg/mL of ATR and 10 μg/mL of AML. The spectra of mixed sample solution was recorded and analyzed by determining the AUC at selected analytical wavelength ranges applying the "Cramer's Rule" and "Matrix Method" (Fig. 5). It is defined as "The total area under curve of a mixture at a particular wavelength range is equal to the sum of area under curve of the individual components at same wavelength range".

$$C^{M} = \frac{X^{N}_{\lambda 1-\lambda 2}AUC_{\lambda 3-\lambda 4} - X^{N}_{\lambda 3-\lambda 4}AUC_{\lambda 1-\lambda 2}}{X^{N}_{\lambda 1-\lambda 2}X^{M}_{\lambda 3-\lambda 4} - X^{N}_{\lambda 3-\lambda 4}X^{M}_{\lambda 1-\lambda 2}} \quad \ldots(1)$$

$$C_{N} = \frac{X^{M}_{\lambda 1-\lambda 2}AUC_{\lambda 3-\lambda 4} - X^{M}_{\lambda 3-\lambda 4}AUC_{\lambda 1-\lambda 2}}{X^{N}_{\lambda 1-\lambda 2}X^{M}_{\lambda 3-\lambda 4} - X^{N}_{\lambda 3-\lambda 4}X^{M}_{\lambda 1-\lambda 2}} \quad \ldots(2)$$

where,

$$\frac{AUC_{\lambda 1-\lambda 2}}{\text{Conc. in g/lit.}} \qquad \frac{AUC_{\lambda 3-\lambda 4}}{\text{Conc. in g/lit.}}$$

Method validation

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies, specificity and reproducibility as per the ICH guidelines[17].

RESULTS AND DISCUSSION

The present manuscript deals with simultaneous estimation of ATR and AML in combined tablet dosage form by area under curve method using methanol as solvent. The developed method is based upon estimation of both the drugs by determining the area under

curve at selected analytical wavelength ranges (256-238.5 nm and 368-352 nm) and solving the Cramer's equation.

The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 5-50 μg/mL for ATR (r = 0.39998) and AML (r = 0.99997) respectively (Table 1).

Table 1: Statistical analysis of the calibration curves of ATR and AML

Parameters	ATR	AML
Range (μg/mL)	5-50	5-50
Slope*	0.0365	0.0113
Intercept*	-0.0032	0.0006
Correlation coefficient (r)*	0.9998	0.9997
LOQ (μg/mL)*	0.5	0.5
LOD (μg/mL)*	0.5	1.0
Where, *n = 6		

Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labeled amount of both the drugs (10 ATR and 5 mg AML) in tablet formulation.

Three replicate samples of each concentration levels were prepared and the percentage recovery at each level (n = 3), and mean % recovery (n = 9) were determined and summarized in Table 2. The mean (%) recovery was found to be 99.83% and 99.60% for ATR and AML, respectively.

Intra-day precision was estimated by assaying the quality control sample of the tablet formulation containing 20 μg/mL of ATR and 10 μg/mL of AML, six times and the results were averaged for statistical evaluation. The statistical validation data for intra day precision is summarized in Table 3.

Table 2: Recovery of ATR and AML in spiked standard drug solution

Level of *(%) Recovery	Amount present (mg)		Amount added (mg)		Amount found (mg)		Recovery** (%)	
	ATR	AML	ATR	AML	ATR	AML	ATR	AML
(80%)	10.0	5.0	8.0	4.0	17.97	8.96	99.83	99.55
(100%)	10.0	5.0	10.0	5.0	19.97	9.98	99.85	99.80
(120%)	10.0	5.0	12.0	6.0	21.96	10.94	99.81	99.45

Where, *n = 3 and **n = 9

Table 3: Statistical validation data for determination of intra-day precision (n = 6)

Interpolated concentration (mean ± SD)*		RSD (%)*	SE (%)*
Atorvastatin calcium (μg/mL) 20	19.92 ± 0.0528	0.2651	0.0215
Amlodipine besylate (μg/mL) 10	9.92 ± 0.0808	0.8145	0.0329

Where, *n = 6

Intra-day precision was evaluated by analyzing a set of quality control samples of the tablet formulation containing 20 μg/mL of ATR and 10 μg/mL of AML, six levels analyzed on three consecutive days. The statistical validation data (results averaged for statistical evaluation) for intra day precision is summarized in Table 4.

Table 4: Statistical validation data for determination of inter-day precision (n = 3)

Interpolated concentration (mean ± SD)*		RSD (%)*	SE (%)*
Atorvastatin calcium (μg/mL) 20	19.91 ± 0.0979	0.4917	0.0565
Amlodipine besylate (μg/mL) 10	9.97 ± 0.0917	0.9197	0.05294

Where, *n = 3

Both intra-day and inter-day variation were found to be showing less % RSD value indicating high grade of precision of the method.

The validation results obtained confirm the suitability of the proposed UV spectrophotometric method for simple, accurate and precise analysis of ATR and AML in pharmaceutical preparations. The proposed method do not need prior separation of ATR and AML before analysis. In addition, the proposed method is suitable for application without interference of excepients and can be applied directly to the commercial preparation without previous treatment.

ACKNOWLEDGEMENT

The authors are heartily grateful to the National Education Society, Shimoga for providing all the facilities to carry out the research work.

REFERENCES

1. S. Budawari, (Ed.) Inc., The Merck Index, 13th Edn., Merck & Co., Inc., Whitehouse Station, New Jersey, (2001) p. 488, 865.
2. S. C. Sweetman, Inc., Martindale, The Complete Drug Reference, 33rd Edn., Pharmaceutical Press, London, (2002) pp. 838-839, 842-843.
3. C. Dollery, Therapeutic Drugs, 2nd Edn., **Vol. 1**, Churchill Livingstone, (1999) p. A151, A228.
4. A. R. Gennaro, (Ed).) Inc., Remington, The Science and Practice of Pharmacy, 20th Ed., **Vol. 1**, Lippincot Williams and Wilkins, New York, (2000) pp. 587-606, 618-620.
5. H. P. Rang, M. M. Dale J. M. Ritter and P. K. More, in, Pharmacology, 5th Edn., Elseiver Science Publisher, (2003) pp. 282-283, p. 310-311.
6. Indian Pharmacopoeia, **Vol. 1**, Government of India, The Controller of Publications, Delhi (1996).
7. The United States Pharmacopoeia, 27th Rev., U. S. Pharmacopoeial Convention, Inc., Rockville, M. D. (2004).
8. British Pharmacopoeia, **Vol. 1**, Her Majesty's Stationary Office, London (2004).
9. R. Klinkenberg. B. Streel and A Ceccato, J. Pharm. Biomed. Anal., **32**, 345-352 (2003).
10. K. R. Naidu, U. N. Kale and M. S. Shingare, J. Pharm. Biomed. Anal., **39**, 147-155 (2005).
11. N. Kamble and R. Venkatachalam, Indian Drugs, **41**, 179-181 (2004).

12. U. J. Dhorda and N. B. Shetkar, Indian Drugs, **36**, 638-641 (1999).

13. U. P. Halkar, N. P. Bhandari and S. H. Rane, Indian Drugs, **35**, 167-168 (1998).

14. K. Manoj and P. Shanmugapandiyan, Indian Drugs, **41**, 284-289 (2004).

15. J. Martin, A. L. Zackrisson and B. Norlander, J. Chromatog., **B. 672**, 310-313 (1995).

16. G. Bahareh, B. Mohammadi, S. Mirzaeei and A. Kiani, J. Chromatog., **B826**, 41-45 (2005).

17. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October, (1994) pp. 1-5.

Revised : 10.04.2009 *Accepted : 14.04.2009*