Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(1): 134-140
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Validated spectrophotometric methods for quantitative determination of Atorvastatin calcium and Metoprolol succinate in Capsules

Sagar B. Wankhede*, Nitin R. Dixit, Sohan S. Chitlange

Padmashree Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, Maharashtra, India

---

Abstract

Three simple, accurate and precise UV-Spectrophotometric methods has been developed and validated for quantitative determination of Atorvastatin calcium (ATS) and Metoprolol succinate (MET) in capsules. First method is based on simultaneous equations and the wavelengths selected for analysis were 246.5 nm (λmax of Atorvastatin calcium) and 276.5 nm (λmax of Metoprolol succinate). Second method involves multicomponent mode of analysis and the wavelengths selected for analysis were same as used in first method. Third method is based on area under curve and the wavelength ranges selected for analysis were 251.5-241.5 nm for Atorvastatin calcium and 281.5-271.5 nm for Metoprolol succinate. Linearity was obtained in the concentration range of 4-24 µg/ml and 10-60 µg/ml for Atorvastatin calcium and Metoprolol succinate, respectively. The results of analysis have been validated statistically and by recovery studies. The utility of the developed methods has been demonstrated by analysis of commercial formulation containing these drugs.

Key Words: Atorvastatin calcium, Metoprolol succinate, simultaneous equation method, multicomponent method, area under curve method.

Introduction

Atorvastatin calcium(ATS), chemically is [R(R*, R*)]-2-(4-flourophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(Phenyl amino) carbonyl]-1H-Pyrrole-1-heptenoic acid, calcium salt (2:1) trihydrate [1]. It is an antihyperlipidemic agent used as HMG-Co-A reductase inhibitor [8]. It is official in IP. Metoprolol succinate (MET), chemically is 1-[4-(2-methoxyethyl)-phenoxy]-3-[(1-methylethylamino]-2-propanol [2]. It is a beta adrenergic blocking agent, which reduces chest pain and lowers high blood pressure [14]. It is official in USP. Literature survey revealed spectrophotometric and chromatographic methods reported for estimation of ATS [3-10] and METO [11-14] individually or in combination with other drugs. However, there is no analytical

method reported for the simultaneous determination of these drugs in a pharmaceutical formulation. Present work describes three simple, rapid, accurate and precise methods for simultaneous determination of ATS and MET in capsules. The proposed methods were validated as per ICH guidelines [15].

Results and Discussion

The methods discussed in the present work provide a convenient and reliable way for quantitative determination of ATS and MET in combined dose capsule formulation. Wavelength of maximum absorbance for ATS (246.5 nm) and MET (276.5 nm) were selected for analysis by simultaneous equation method (Method I) and multicomponent mode of analysis (Method II). In area under curve method (Method III) quantitative determination was carried out at wavelength range 251.5-241.5 nm (for ATS) and 281.5-271.5 nm (for MET). Linearity for ATS and MET was observed in the concentration range of 4-24 µg/ml and 10-60 µg/ml, respectively, for all three methods. Percent label claim for ATS and MET in capsule analysis, by all the methods, was found in the range of 100.23 to 100.75 %. Percent recovery for ATS and MET, by all the methods, was found in the range of 99.62 % to 101.54 % with standard deviation well below 2 indicating accuracy of the methods. Intra-day and Inter-day precision studies were carried out by analyzing capsule formulation, by all the methods, three times on the same day and on three different days, respectively. Standard deviation and coefficient of variance for intra-day and inter-day precision studies was satisfactorily low indicating high degree of precision and reproducibility of proposed methods.

Material and Methods

Standard gift samples of Atorvastatin calcium and Metoprolol succinate were provided by Mcleods Pharmaceuticals Ltd, Mumbai and Emcure Pharmaceuticals Ltd, Pune respectively. Combined dose capsule formulation containing Atorvastatin calcium (10 mg) and Metoprolol succinate (25 mg), manufactured by Dr. Reddy's Laboratory, were purchased from local market. Methanol- AR was used as solvent.

A double-beam Shimadzu UV- Visible spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of solution.

Preparation of Standard Stock Solution: Accurately weighed quantity of ATS/MET (5 mg) was transferred to 50.0 ml volumetric flask, dissolved and diluted to the mark with methanol. (Concentration: 100 µg/ml).

Method I: Simultaneous Equation Method

For the selection of analytical wavelength, standard solution of ATS (20 µg/ml) and MET (50 µg/ml) were prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the entire UV range to determine λ_{max} of both the drugs. The λ_{max} of ATS and MET were found to be 246.5 nm and 276.5 nm, respectively. A series of standard solutions were prepared having concentration range of 4-24 µg/ml for ATS and 10-60 µg/ml for MET. The absorbance of resulting solutions was measured at 246.5 nm and 276.5 nm and

calibration curves were plotted. Both the drugs obeyed linearity in the concentration range under study. Absorptivity values were then determined for both the drugs at selected wavelengths. Two simultaneous equations (in two variables C_1 and C_2) were formed using absorptivity coefficient values obtained and are as follows:

$$A_1 = 30.11C_1 + 18.07C_2 \quad (1)$$
$$A_2 = 2.88C_1 + 6.46C_2 \quad (2)$$

Where A_1 and A_2 are the absorbance of sample solution at 246.5 nm and 276.5 nm, respectively. C_1 and C_2 are the concentrations of ATS and MET measured in mg/ml, in sample solutions. Absorptivity values 30.11 & 18.07 are of ATS at 246.5 nm and 276.5 nm, respectively. Similarly, 2.88 & 6.46 are absorptivity values of MET at 246.5 nm and 276.5 nm, respectively. By applying the Cramer's rule to equation 1 and 2, the concentration C_{ATO} and C_{METO}, can be obtained as follows,

$$C_{ATO} = \frac{A_2(2.8875) - A_1(6.4604)}{-142.469} \quad (3)$$

$$C_{METO} = \frac{A_1(18.07) - A_2(30.11)}{-142.469} \quad (4)$$

Method II: Multicomponent Mode of Analysis

In this method, six mixed standard solutions with concentration of ATS and MET in the ratio of 8:20 µg/ml were prepared in methanol. All the standard solutions were scanned in the entire UV range, in the multicomponent mode, using two working wavelength 246.5 nm (λ_{max} of ATS) and 276.5 nm (λ_{max} of MET). The data from these scans was used to determine the concentrations of two drugs in capsule sample solutions.

Method III: Area Under Curve Method

From the overlain spectra of drugs (Fig.1), area under the curve in the range of 251.5-241.5 nm (for ATS) and 281.5-271.5 nm (for MET) were selected for the analysis. The calibration curves for ATS and MET were plotted in the concentration range of 4-24 µg/ml and 10-60 µg/ml, respectively. The 'X' values for both the drugs were determined at the selected AUC range. The 'X' value is the ratio of area under the curve at selected wavelength ranges with the concentration of component in g/lit. A set of two simultaneous equations obtained by using mean 'X' values are as follows:

$$A_1 = 301.10C_1 + 188.3C_2 \quad (\text{at } \lambda_{251.5\text{-}241.5}\text{nm}) \quad (5)$$
$$A_2 = 30.30C_1 + 59.80C_2 \quad (\text{at } \lambda_{281.5\text{-}271.5}\text{nm}) \quad (6)$$

Where A_1 and A_2 are area under curve of sample solution at the wavelength ranges 251.5-241.5 nm and 281.5-271.5 nm, respectively. The 'X' values 301.10 and 188.3 are of ATS at wavelength range 251.5-241.5 nm and 281.5-271.5 nm, respectively. Similarly, 30.30 and 59.80

are 'X 'values of MET at wavelength range 251.5-241.5 nm and 281.5-271.5 nm, respectively. The concentration of ATS and MET in sample solution was determined by using the equations (5) and (6).

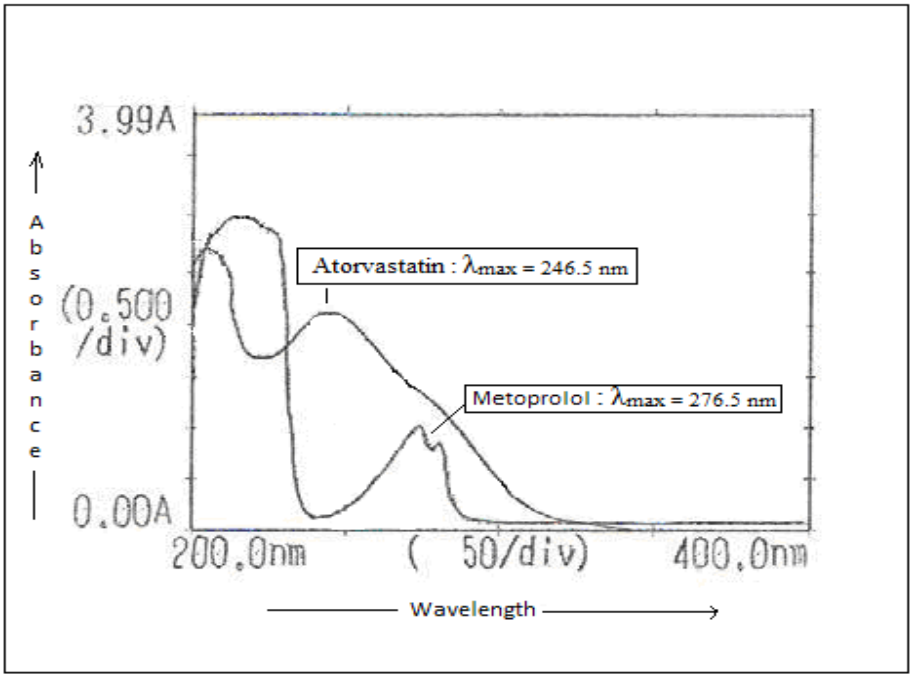

Fig.1: Overlain spectra of Atorvastatin calcium and Metoprolol succinate

Analysis of Capsule Formulation by Proposed Methods

For the estimation of drugs in the commercial formulation, twenty capsules were weighed and the capsule powder was removed. The weight of empty capsule shells was then recorded. Difference in the weight of filled capsules and empty capsule shells was calculated to know the weight of powder present in twenty capsules. Average weight was then calculated. Capsule powder equivalent to about 10 mg ATS and 25 mg of MET was transferred to 25.0 ml volumetric flask; 10 ml methanol was added and sonicated for 20 min., volume was then made up to the mark with methanol. The resulting solution was mixed and filtered through Whatmann filter paper and filtrate was appropriately diluted to get approximate concentration of 8 µg/ml of ATS and 20 µg/ml of MET. In method I, concentration of both ATS and MET were determined by measuring absorbance of sample solution at 246.5 nm & 276.5 nm and using equations (3) and (4). In method II, the sample solution was subjected to analysis in the multicomponent mode of instrument, concentration of both ATS and MET was determined by analysis of spectral data of the sample solution with reference to the mixed standards at 246.5 nm and 276.5 nm. In method III, concentration of both ATS and MET was determined by measuring area under curve in the range of 251.5-241.5 nm (for Atorvastatin calcium.) and 281.5-271.5nm (for Metoprolol succinate) and values were substituted in equations (5) and (6) to obtain concentration of both the drugs. Results of capsule analysis are shown in Table No. 1.

Table No – 1: Result of Analysis of Capsules

Method	Drug	Label Claim (mg/capsule)	Amount of drug estimated* (mg/capsule)	% of label claim estimated, ± S.D*
I	ATS	10	10.07	100.75 ± 0.59
	MET	25	25.18	100.71 ± 0.79
II	ATS	10	10.04	100.41 ± 0.57
	MET	25	25.04	100.16 ± 0.25
III	ATS	10	10.02	101.23 ± 0.17
	MET	25	25.15	100.59 ± 0.42

* Mean of six determinations. ATS =Atorvastatin calcium, MET= Metoprolol succinate

Validation

The proposed methods were validated as per ICH guidelines.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% &120%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table No.2.

Table No – 2: Result of Recovery Studies

Level of recovery	Amount of Pure Drug added (mg)		Percent Recovery*					
			Method-I		Method-II		Method-III	
	ATS	MET	ATS	MET	ATS	MET	ATS	MET
80	8	20	100.62	100.16	100.78	100.26	99.90	100.00
100	10	25	99.93	100.27	101.01	99.62	100.23	99.98
120	12	30	99.84	100.24	101.54	99.73	99.69	99.99
Mean % recovery			100.13	100.22	101.11	99.87	99.94	99.99
S. D.			0.4267	0.0568	0.3897	0.3422	0.2722	0.0100
C. V.			0.4261	0.0566	0.3854	0.3426	0.2723	0.0100

*mean of three determinations, ATS- Atorvastatin calcium, MET- Metoprolol succinate, SD- Standard Deviation, CV- Coefficient of Variance

Precision

The reproducibility of the proposed methods was determined by analyzing capsules at different time intervals on same day (Intra-day assay precision) and on three different days (Inter-day assay precision). Coefficient of variance for intra-day assay precision was found to be 0.1709 (for Atorvastatin calcium.) & 0.5814 (for Metoprolol Succinate) in simultaneous equation method, 0.2643 (for Atorvastatin calcium.) & 0.1616 (for Metoprolol Succinate) in multicomponent mode of analysis and 0.0057 (for Atorvastatin calcium.) & 0.0458 (for Metoprolol Succinate) in area under curve method. Inter-day assay precision coefficient of variance was found to be 0.1708 (for Atorvastatin calcium.) & 0.0577 (for Metoprolol Succinate) in simultaneous equation method, 0.2643 (for Atorvastatin calcium.) & 0.0831 (for Metoprolol Succinate) in multicomponent mode of analysis and 0.0575 (for Atorvastatin calcium.) & 0.2524 (for Metoprolol Succinate) in area under curve method.

Conclusion

Based on the results obtained, it can be concluded that the proposed UV-Spectrophotometric methods (simultaneous equation method, multicomponent mode of analysis and area under curve method) for simultaneous determination of Atorvastatin calcium and Metoprolol succinate are rapid, economical, accurate and precise. The utility of the developed methods has been demonstrated by analysis of combined dose capsule formulation. Hence, the proposed methods can be used for quantitative determination of pharmaceutical formulation containing these ingredients in combination.

Acknowledgement

The authors are thankful to Dr. Avinash D. Deshpande, Director of Pharmacy, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for providing necessary facilities. Authors are also thankful to Mcleods Pharmaceuticals, Mumbai and Emcure pharmaceuticals, Pune for providing gift sample of pure drugs.

References

[1] Indian Pharmacopoeia, published by the controller of publication, New Delhi, **2007**, II, 749
[2] United States Pharmacopoeia, XXVI, National Formulary XXI, Asian edition, US Pharmaceutical Convention, Inc; Rockville, MD **2003**, 1220
[3] P. Nagaraju, Pasumarthy, N.V. Gopal, V.D.N. Srinivas and S.V.N. Padma, *Asian Journal of Research in Chemistry*, **2008**, 1(2), 64-66.
[4] V.P. Godse, M.N.Deodhar, A.V. Bhosale, R.A. Sonawane, P.S. Sakpal, D.D Borkar, *Asian Journal of Research in Chemistry* **2009,** 2(1),86-89.
[5] R.G. Baldha, V.B. Patel, M. Bapna, *International Journal of ChemTech Research* **2009,** 1(2), 233-236
[6] Zahid Zaheer, M.N. Farooqui, A.A. Mangle, A.G. Nikalje, *African Journal of Pharmacy and Pharmacology* **2008,** 2(10), 204-210.
[7] S.S. Qutab, S.N. Razzaq, I. Khan, Md. Ashfaq, Z.A. Shuja, *Journal of food and drug analysis* **2007,** 15(2), 139-144.

[8] S.S. Dhaneshwar, S.R. Dhaneshwar, P. Deshpande, M. Patil, *Acta Chromatographica* **2007**, 19, 141-148
[9] Jamshidi, R. Nateghi, *Chromatographia* **2007,** 65(11/12), 763–766
[10] B.G. Chaudhari, N.M. Patel, P.B.Shah, K.P. Modi, *Indian J. Pharm Sci*. **2006,** 68(6), 793-796.
[11] M.N. Kulkarni, R.V. Kshirsagar, D.M. Sakarkar, *International Journal of Chemtech Research* **2009,** 1(4), 1273-1277.
[12] S.S. Chitlange, I. Mohammed, D.M. Sakarkar, *Asian Journal of Pharmaceutics* **2008**, 232-4
[13] M.D. Phale, P.D. Hamrapurkar, *Asian Journal of Research in Chemistry* **2009,** 2(2), 119-22.
[14] V.G. Dongre, S.B. Shah, P.P. Karmuse, M. Phadke, V.K. Jadhav, *Journal of Pharmaceutical and Biomedical Analysis* **2008**,46, 583–586.
[15] Validation of Analytical Procedures: Text and Methodology, Proceedings of International Conference on Harmonization (ICH). Geneva, **2005**