Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011, 3(1): 1-7
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Stability Indicating HPTLC method for Quantitative Determination of Atorvastatin calcium and Metoprolol succinate in Capsules

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

Padmashree Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Sant Tukaram Nagar, Pimpri, Pune- 411018, Maharashtra, India.

ABSTRACT

A simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) method has been developed and validated for analysis of Atorvastatin calcium and Metoprolol succinate in capsules. Both the drugs were separated on aluminum-backed silica gel 60 F_{254} plates with Toluene: methanol: ethyl acetate: glacial acetic acid (7: 1.5: 1: 0.5 v/v/v/v) as mobile phase. This system was found to give compact bands for Atorvastatin calcium and Metoprolol succinate (R_F values 0.28 ± 0.1 and 0.58 ± 0.1, respectively). Densitometric analysis of Atorvastatin calcium and Metoprolol succinate was performed at 276 nm. Regression analysis data for the calibration plots were indicative of good linear relationships between response and concentration over the range 500–3000 ng per band for Atorvastatin calcium and 1000-6000 ng per band for Metoprolol succinate The correlation coefficients, r^2, were 0.9974 and 0.9927 for ATS and MET calibration curve, respectively. The method was validated as per ICH guidelines for accuracy, precision, LOD, LOQ and robustness. Atorvastatin calcium and Metoprolol succinate were subjected to acid, base, peroxide, heat and UV-induced degradation.

Key Words: Atorvastatin calcium, Metoprolol succinate, HPTLC, Degradation

---

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 simple, rapid, accurate and precise High Performance Thin Layer Chromatographic method for simultaneous determination of ATS and MET in capsules. The proposed method was validated as per ICH guidelines [15].

MATERIALS AND METHODS

Materials: Standard gift samples of Atorvastatin calcium and Metoprolol succinate were provided by Macleods 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, was purchased from local market. Methanol- AR was used as solvent.

Instrumentation and chromatographic conditions:
The sample was applied as 6 mm wide bands, under a continuous flow of nitrogen, using Camag Linomat V (Muttenz, Switzerland) sample applicator fitted with a 100 mcL syringe (Hamilton, Bonaduz, Switzerland) on 10 cm x 10 cm aluminum-backed HPTLC plates coated with silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). A constant application rate of 150 nL s^{-1} was used and the distance between adjacent bands was 5.6 mm. The plates were prewashed with methanol and activated prior to chromatography. Linear ascending development of the plates to a distance of 80 mm was performed with Toluene: methanol: ethyl acetate: glacial acetic acid (7: 1.5: 1: 0.5 v/v/v/v), as mobile phase in a twin-trough glass chamber (10-10 cm^2) (Camag, Muttenz, Switzerland) previously saturated with mobile phase for 10 min at room temperature (25°C). After chromatographic development, plates were dried in the air and densitometric scanning was performed at 276 nm with a Camag TLC scanner III operated in reflectance-absorbance mode and controlled by WinCATS software (Version 1.4.3.6336). The slit dimensions were 5 x 0.45 mm and the scanning speed was 20 mm s^{-1}.

Method development
A variety of mobile phases were investigated to establish a suitable HPTLC method for analysis of ATS and MET in bulk drug and capsule dosage form. These included chloroform-methanol, 5:5 (%, v/v), chloroform- ethyl acetate, 6:4 (%, v/v), chloroform-methanol–acetone, 3:3:4 (%, v/v), and ethyl acetate-methanol, 7.5:2.5 (%, v/v). The suitability of the mobile phase was decided by study of the sensitivity of the assay, the time required for analysis, and the use of readily available solvents.

Preparation of Standard Stock Solutions and linearity studies
A fresh standard stock solution of 500 mcg mL^{-1} ATS was prepared in methanol. Aliquots of standard stock solution (1, 2, 3, 4, 5 and 6 mcL) were applied to a TLC plate to furnish final amounts of 500-3000 ng per band. Similarly a fresh standard stock solution of 1000 mcg mL^{-1} MET was prepared in methanol. Aliquots of standard stock solution (1, 2, 3, 4, 5 and 6 mcL) were applied to a TLC plate to furnish final amounts of 1000-6000 ng per band. The plate was

chromatographed and scanned under the above mentioned chromatographic conditions. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Analysis of Marketed Formulation

To determine the content of ATS and MET in combined dose capsule formulation (Brand name: Betaone-ATR, label claim: 10 mg Atorvastatin calcium and 25 mg Metoprolol succinate per capsule), twenty capsules were weighed; powder from the capsules was removed and the empty capsule shell were reweighed. Difference in the weight expressed weight of powder present in twenty capsules. Average weight was calculated and capsule powder equivalent to about 15 mg ATS and 37.5 mg MET was transferred to 25.0 mL volumetric flask, 20.0 mL of methanol was added and content of the flask were ultrasonicated for 10 minutes, volume was then made up to the mark with methanol. The solution was mixed and filtered through Whatman filter paper No. 42. From the filtrate, 5.0 mL was diluted to 10.0 mL with methanol. On TLC plate two bands of standard and four bands of sample solution, 5 µL each, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the peaks obtained for standard and sample bands were integrated. Amount of the drugs present in applied volume of sample solution was obtained by comparison between peak area of standard and sample bands. Six samples were prepared and analyzed in similar manner. Results of analysis of capsules are shown in Table No. 1

Table No – 1: Result of Analysis of Capsules

Component	Label claim	Amount found*	Percent label claim*	S. D.	R. S. D.
ATS	10	10.005	100.50	± 0.3507	0.3505
MET	25	24.921	99.69	± 0.6870	0.6891

Method validation

Accuracy

Recovery was determined to confirm the accuracy and suitability of the method. Recovery study was carried out by standard addition method. ATS and MET corresponding to 80, 100 and 120% of label claim had been added to the preanalysed capsule sample and the mixtures were analyzed by the proposed method. At each level of recovery three determinations were performed. Results of the recovery studies are shown in Table No. 2.

Precision

In accordance with ICH recommendations precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability of sample application was determined as intraday variation whereas intermediate precision was determined by measuring interday variation for triplicate determination of ATS and MET at concentrations 1500 and 3750 ng per band of ATS and MET, respectively.

Robustness

Robustness of the method was studied by deliberately making small changes in mobile phase composition (± 0.1 mL), volume of the mobile phase (± 1 mL) and by varying saturation time (± 10 %). The effect of varied conditions on the retention factor and peak shapes was examined.

Limits of Detection (LOD) and Quantification (LOQ)

ATS and MET at concentrations in the lower part of the linear range of the calibration plot were used to determine limit of detection (LOD) and limit of quantification (LOQ). They were determined from the slope of the calibration plot and standard deviation (SD) of the blank sample by use of the equations:

$$LOD = 3.3 \times SD/S \text{ and } LOQ = 10 \times SD/S$$

Where, SD is the standard deviation of the blank response and S is the slope of the calibration plot.

Forced Degradation study of ATS and MET

Amount of capsule powder equivalent to about 15 mg ATS and 37.5 mg MET was separately transferred to five different 50.0 mL volumetric flasks (Flask No. 1, 2, 3, 4 and 5), added 1 mL of 0.1 M HCl, 0.1 M NaOH and 3 % H_2O_2 to Flask No. 1, 2 and 3, respectively. Contents in the Flask No. 1, 2, and 3 were heated in water bath at 80^{o}C for 3 hrs. Flask No. 4 containing capsule powder was kept at 60^{0}C for 24 hrs to study the effect of heat on capsule sample (heat degradation). The forced degradation was performed in the dark to exclude the possible degradative effect of light. Flask No. 5 was exposed to ultraviolet radiations at 276 nm for 24 hrs in a UV-chamber. All the flasks were removed after respective time interval; the capsule samples were treated and analyzed in similar manner as described under analysis of marketed formulation.

RESULT AND DISCUSSION

Optimization of the method:

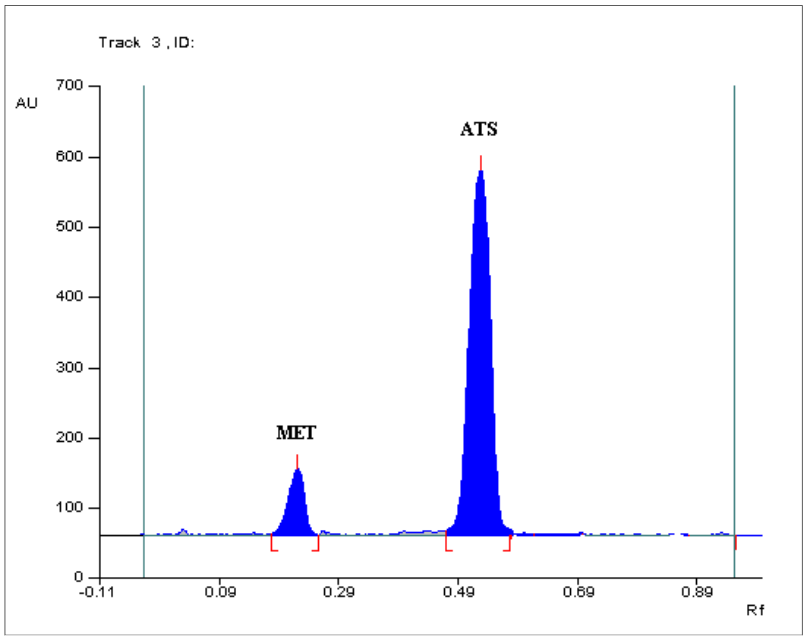

Fig. No. 1: Typical Densitogram of Atorvastatin calcium andMetoprolol succinate

HPTLC procedure was optimized to develop a stability-indicating assay. The mobile phase Toluene: methanol: ethyl acetate: glacial acetic acid (7: 1.5: 1: 0.5 v/v/v/v) resolved ATS and MET with R_F values of 0.28 ± 0.1 and 0.58 ± 0.1, respectively (Fig. No. 1). Well defined bands (peaks) were obtained when the chamber was saturated with mobile phase vapor for 10 min at room temperature (25°C).

Linearity:
Linear regression data for the calibration plots for ATS and MET were indicative of a good linear relationship between peak area response and concentration over the range 500-3000 ng per spot for ATS and 1000-6000 ng per spot for MET with the regression coefficients 0.9974, for ATS and 0.9927, for MET.

Accuracy:
The accuracy was expressed as the percentage of analytes recovered by the assay method. The percent recovery was found in the range of 98.81 – 100.17 % for both the drugs (Table No. 2), which indicated the accuracy of the method was adequate. The low value of % RSD indicated the method was suitable for routine analysis of the ATS and MET in pharmaceutical dosage forms.

Table No – 2: Result of Recovery Studies

Level of recovery	Amt. of pure drug added		Percent recovery*	
	ATS	MET	ATS	MET
80 %	12.0	30.0	99.74	98.81
100 %	15.0	37.5	99.64	99.19
120 %	18.0	45.0	99.48	100.17
		Mean % recovery **	99.62	99.39
		S.D.	± 0.3222	± 0.7354
		R.S.D.	0.3234	0.7399

Precision:
The relative standard deviation (R.S.D) values for intra-day and inter-day precision for ATS were 0.0871 % and 0.4507 %, respectively and that for MET were 0.1319 and 0.2051, respectively which are well within the acceptable limit of 2 %

Robustness:
The low values of % RSD in the results obtained after introducing small changes in mobile phase composition, volume, and saturation time were indicative of the robustness of the method. There was no significant variation of the slopes of the calibration plots.

Limits of Detection (LOD) and Quantification (LOQ):
The LOD and LOQ were determined from the slope of the lowest part of the calibration plot. The LOD and LOQ were 15.001 and 45.457 respectively, for ATS and 78.736 and 238.595 ng, respectively, for MET, which indicates the sensitivity of the method is adequate.

Forced Degradation of ATS and MET:
The chromatograms obtained from samples degraded by treatment with acid, base, hydrogen peroxide, heat, and UV light contained some additional peaks at different R_F values. It is

apparent from Figs 2–6 that peaks of ATS and MET were not significantly shifted in the presence of the degradation peaks, which indicated the stability-indicating nature of the method.

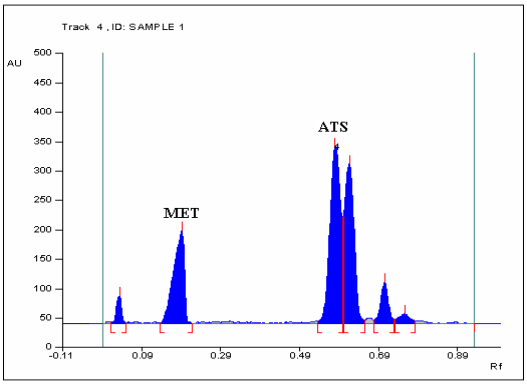

Fig. No. 2: Chromatogram of 0.1 M HCl treated capsule sample

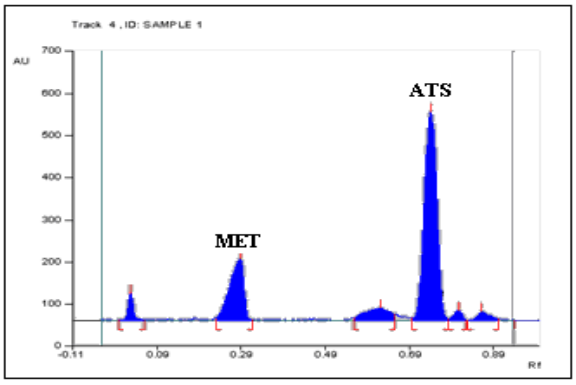

Fig. No. 3: Chromatogram of 0.1 M NaOH treated capsule sample

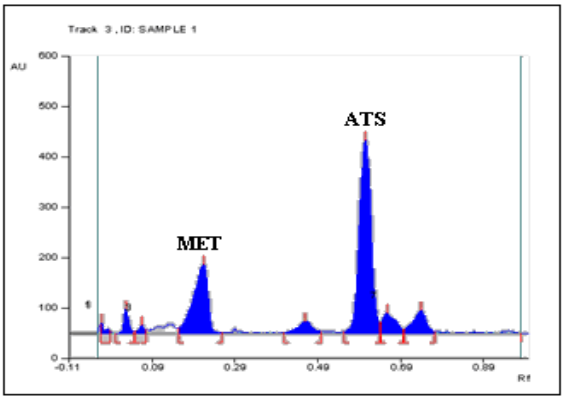

Fig. No. 4: Chromatogram of H_2O_2(3 %) treated capsule sample

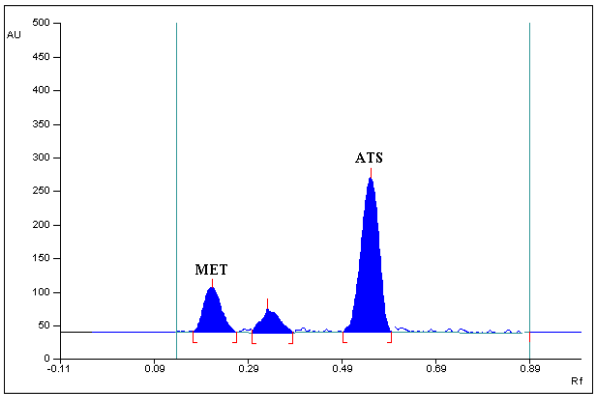

Fig. No: 5: Chromatogram of dry heat treated capsule sample

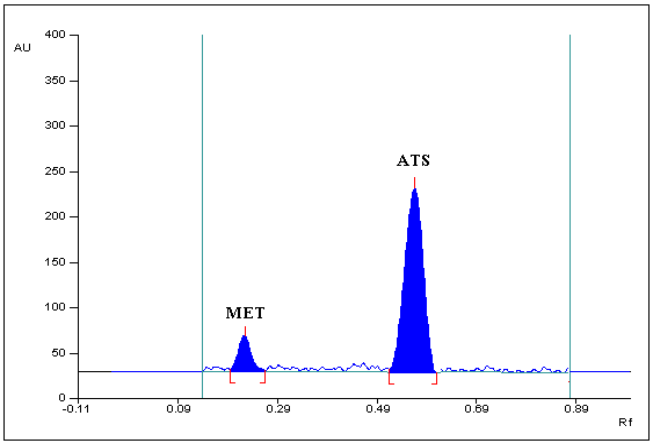

Fig. No. 6: Chromatogram of UV radiation treated capsule sample

CONCLUSION

This HPTLC method is precise, specific, accurate, and stability indicating. Statistical analysis proved the method is repeatable and selective for the analysis of Atorvastatin calcium and Metoprolol succinate in pharmaceutical formulations. The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities.

Acknowledgement
The authors express their gratitude to Dr. A. D. Deshpande, Director, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, Maharashtra, India, for providing necessary facilities, and to Macleods Pharmaceuticals, Mumbai, and Emcure Pharmaceuticals, Pune for the generous gift samples of pure Atorvastatin calcium and Metoprolol succinate.

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 The International Conference on Harmonization (ICH). Geneva, **2005**