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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF METOPROLOL SUCCINATE AND ATORVASTATIN CALCIUM IN A PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new, simple, precise, accurate and selective high performance thin-layer chromatographic (HPTLC) method has been developed and validated for the simultaneous determination of Metoprolol succinate and Atorvastatin calcium in a marketed formulation. Chromatographic separation was carried out on Merck TLC aluminium sheets of silica gel $60F_{254}$ using Acetonitrile: Methanol: Ethyl acetate: Glacial acetic acid (2: 4: 4: 0.06 % v/v/v/v) as mobile phase followed by densitometric analysis at 223 nm. This system was found to give compact spots for Metoprolol succinate (Rf value of 0.32 ± 0.005) and Atorvastatin calcium (Rf value of 0.77 ± 0.004). The method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and specificity in accordance with International Conference on Harmonization (ICH) guidelines. The calibration curve was found to be linear between 500 to 3000 and 200 to 1200 ng/spot for Metoprolol succinate and Atorvastatin calcium, respectively with significantly high value of correlation coefficient (r^2 > 0.99). The limits of detection and quantitation were found to be 0.8432 and 2.5553 ng/spot, respectively for Metoprolol succinate and 27.8428 and 84.3662 ng/spot, respectively for Atorvastatin calcium. The proposed method was found to be accurate, precise, reproducible, specific and sensitive and can be applicable for the simultaneous determination of Metoprolol succinate and Atorvastatin calcium in marketed formulation.

KEY WORDS Metoprolol succinate, Atorvastatin calcium, HPTLC, simultaneous determination, validation.

INTRODUCTION

Metoprolol succinate (MS) is chemically Bis[(2*RS*)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] butanedioate (Figure 1).It is a antihypertensive drug[1,2]. It is official in USP,EP, BP Pharmacopoiea[1,2,3]. It is assayed by Liquid chromatography as per USP and assayed by potentiometric method as per BP and EP. Literature review reveals HPLC, and UV spectrophotometric for estimation of MS in pharmaceutical dosage form[4-9] . Atorvastatin calcium (ATR) is (β*R*,δ*R*)-2-(4-Fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid calcium salt (Figure 2) used as Lipid Lowering Agent[10].It is official in IP and is estimated by liquid chromatography method as per IP.Literature review reveals HPLC, UV spectrophotometric and HPTLC method for the estimation of ATR in pharmaceutical dosage form[11-17] and one HPLC,one HPTLC and one UV spectroscopy with MS[18,19,20]. The present developed method is new, simple, precise, accurate and selective for simultaneous determination of both drugs in their marketed formulation as per International Conference on Harmonization (ICH) guidelines[21].

EXPERIMENTAL

Material and reagents

Metoprolol succinate was kindly supplied by CTX Life Sciences Pvt. Ltd.,Surat,Gujarat,India as gratis sample and Atorvastatin calcium was obtained from Torrent pharmaceuticals,Ahmedabad,Gujarat,India.Acetonitrile,Ethyl acetate,Methanol,and Glacial acetic acid were used as solvents to prepare the mobile phase. All reagents used were of analytical reagent grade. (Methanol from Allied Chemical Corporation, Vadodara and Acetonitrile,Ethyl acetate from SDFCL Pvt. Ltd.,Maharatra and Glacial acetic acid from Chemdyes Corporation,Rajkot). The tablet samples were obtained from local market (Metpure – ST from Emcure Pharmaceuticals Ltd.).

Instrumentation and chromatographic conditions

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (20 × 10 cm), a derivatization chamber, and a plate heater. Pre-coated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were pre-washed twice with 10 mL of methanol and activated at 80^0C for 5 min prior to sample application. The standard and formulation samples of MS and ATR in mixture were spotted on Pre-coated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s. The mobile phase consists of Acetonitrile: Methanol: Ethyl acetate: Glacial acetic acid (2: 4: 4: 0.06 % v/v/v/v) . Linear ascending development was carried out in twin trough chamber (10 x 10 cm). The optimized chamber saturation time for mobile phase was 30 min, at 25^0C ± 2; the length of chromatogram run was 70 mm and TLC plates were air dried. Densitometric scanning was performed on CAMAG TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 223 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. The parameters were selected as recommended by the CAMAG TLC scanner III manual. Evaluation was performed using linear regression analysis via peak areas.

Standard solutions and calibration curves

Standard stock solution of combined drugs was prepared containing 0.025 g/L of MS and 0.010 g/L of ATR in methanol. Which were further diluted with methanol to obtain 250µg/mL of MS and 100 µg/mL of ATR. Calibration was done by applying mixture of standard solutions ranging from 2.0 – 12.0 µL by Hamilton syringe with the help of Linomat V autosprayer on TLC plate that gave concentration

500-3000 ng/spot for MS and 200-1200 ng/spot for ATR, respectively. Each concentration was spotted six times on TLC plates. From the developed plates calibration curve was plotted as peak areas versus corresponding concentrations (Figure 5 and 6).

Method Validation

HPTLC method development

In trial phase Acetonitrile and methanol in ratio of 3:7 (v/v) was used and peak shape is good but it shows high R_f value of ATR. Then Ethyl acetate was incorporated to the mobile phase composition and tried with (2:4:4 % v/v/v) ratio and separation is good but tailing is observed. Then Glacial acetic acid is incorporated to the mobile phase composition and tried with Acetonitrile:Methanol:Ethyl acetate:Glacial acetic acid (2:4:4:0.06 % v/v/v/v) ratio shows good resolution.Both the peaks were symmetrical in nature and no tailing was observed when plate was scanned at 223 nm. The chamber was saturated with the mobile phase for 30 min at room temperature.

Linearity

Linearity responses for MS and ATR were assessed in the concentration range 500-3000 ng/spot and 200-1200 ng/spot of standard solutions, respectively.

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (MS and ATR) was spiked at three different levels. These solutions were subjected to re-analysis by the proposed method.

Sensitivity

The sensitivity of measurement of MS and ATR by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation.

Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently as shown in Figure 7. The spot for MS and ATR was confirmed by comparing the R_f and spectra of the spot with that of standard. The wavelength 223 nm for detecting peak purity of MS and ATR was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Repeatability

Repeatability of sample application was assessed by spotting 8µL (2000 ng/spot of MS and 800 ng/spot of ATR) of drug solution six times on a TLC, followed by development of plate and recording the peak area for six spots.

Analysis of MS and ATR in marketed formulation

To determine the content of MS and ATR simultaneously in conventional tablets (label claim 23.75 mg MS equivalent to Metoprolol tartrate 25 mg and 10 mg ATR); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 23.75 mg (MS) and 10 mg (ATR) was transferred into 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to mark with same solvent. The solution was filtered through 0.45 µm filter (Millifilter, MA) and the volume was adjusted up to mark with methanol to get a final concentration of MS (237.5 µg/mL) and ATR (100 µg/mL). 8µL of this solution applied on TLC plate followed by development and scanning & the analysis was repeated for three times.

Table 1: Result of Calibration reading for MS and ATR

Conc. (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD	Conc. (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD
500	0.32	752.4167±8.1283	1.0802	200	0.77	2512.4± 13.056	0.5196
1000	0.32	1251.0166±12.120	0.9688	400	0.77	2867.95±18.992	0.6622
1500	0.31	1956.3833±15.274	0.7807	600	0.77	3331.88±20.552	0.6168
2000	0.31	2700.75±40.9338	1.5156	800	0.78	3810.58±16.307	0.4279
2500	0.32	3367.3±22.3315	0.6631	1000	0.77	4242.26±15.514	0.3671
3000	0.32	3936.735±18.2127	0.4626	1200	0.77	4695.48±10.945	0.2331

Table 2: Statistical Data of MS and ATR

Parameters	Results	
	MS	ATR
Linear Range(ng/spot)	200-1200	500-3000
Slope	1.3151	2.2167
Intercept	25.9528	2025.05
Std. Deviation of Slope	0.0070	0.0072
Std. Deviation of Intercept	0.3360	18.7017
Limit of Detection(ng/spot)	0.8432	27.8408
Limit of Quantification(ng/spot)	2.5553	84.3662
Regression Equation	y = 1.3202x + 26.08	y = 2.2149x + 2015.5
Co-Relation Co-Efficient (r)	0.9987	0.9993
Co-Efficient of Determination (r^2)	0.9976	0.9987

Table 3: Intra Day and Inter Day study of MS

Concentration (ng/spot)	Intra Day Area Mean (n=3) ± SD	%RSD	Inter Day Area Mean (n=3) ± SD	%RSD
1500	1954.73±27.0853	1.386	1968.47±23.1865	1.178
2000	2705.83±18.279	0.676	2718.63±24.0928	0.886
2500	3347.47±36.4529	1.089	3347.63±28.2642	0.844

Table 4: Intra Day and Inter Day study of ATR

Concentration (ng/spot)	Intra Day Area Mean (n=3) ± SD	%RSD	Inter Day Area Mean (n=3) ± SD	%RSD
600	3339.57±33.5822	1.006	3347.4±42.6858	1.275
800	3829.43±27.836	0.727	3809.57±21.2806	0.559
1000	4228±27.1365	0.642	4246.67±21.5013	0.742

Table 5: Determination of Accuracy for MS and ATR

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
MS 950	475	1425	1417.60	99.48	0.2260
	950	1900	1908.02	100.42	0.2933
	1425	2375	2379.55	100.19	0.1026
ATR 400	200	600	598.70	99.78	0.1599
	400	800	794.08	99.26	0.2032
	600	1000	1009.66	100.97	0.3084

Table 6: Repeatability study of MS and ATR

Concentration	MS (800 ng/spot)	ATR (2000 ng/spot)
Area	2702.2	3814.5
	2710.1	3836.1
	2699.2	3845.1
	2714.3	3825
	2703	3805
	2768.5	3820
Mean	2716.21	3824.283
± SD	26.2120	14.5560
%RSD	0.9650	0..3806

Table 7: Assay Result of marketed formulation

Formulation	Metpure – ST	
	MS	ATR
Actual Concentration (ng/spot)	1900	800
Concentration Obtained (ng/spot)	1908.2045	802.2865
%Purity	100.43	100.29
%RSD	0.669	0.518
Limit[2,10]	99.0% -101%	98.0 % -102.0%

Table 8: Validation Parameters

Summary of Validation Parameters		
	MS	ATR
Recovery (%)	99.48-100.42	99.78-100.97
Repeatability (%RSD)	0.899	0.996
Precision (CV) Intra-day (n=3)	0.0105	0.00791
Inter-day (n=3)	0.00969	0.00859
Specificity	Specific	Specific
Selectivity	Selective	Selective

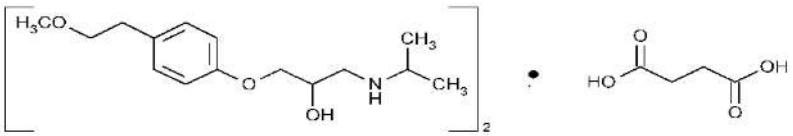


Figure 1: Structure of Metoprolol succinate

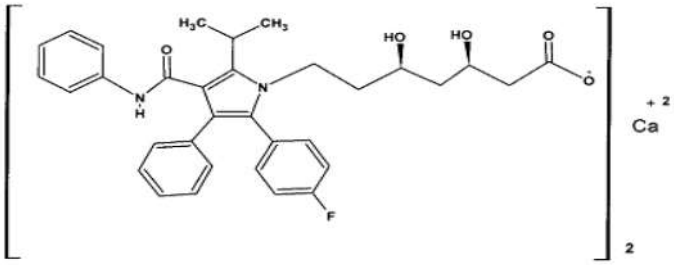


Figure 2: Structure of Atorvastatin calcium

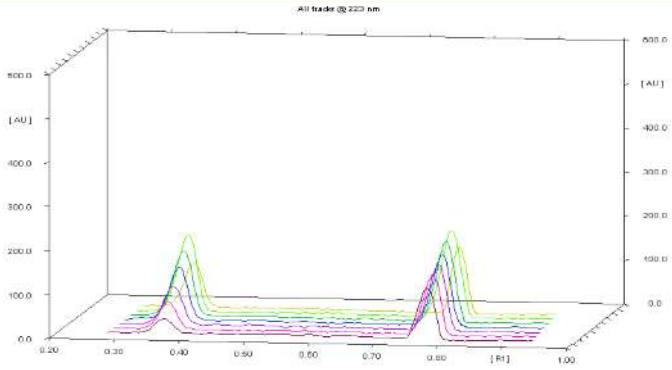


Figure 3: 3D Representation of Densitogram for Calibration curve of MS and ATR

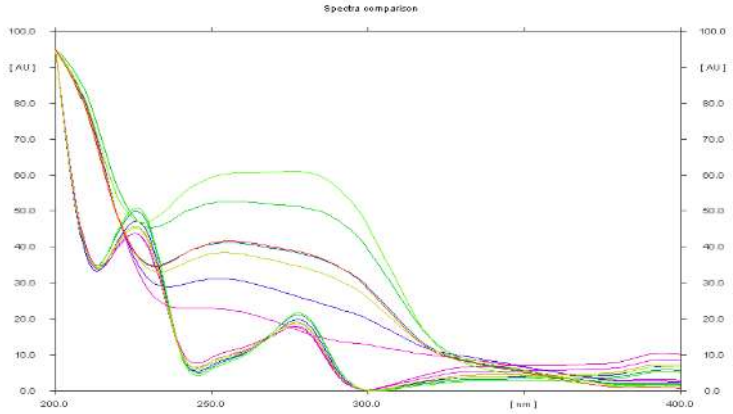


Figure 4: Overlay UV Absorption (Reflectance Mode) of the corresponding spots for MS and ATR

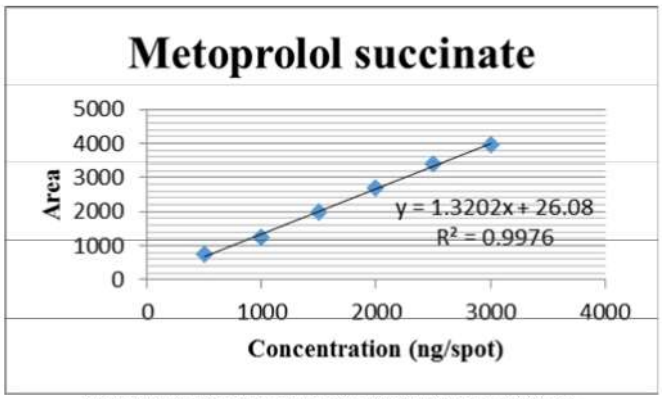


Figure 5: Calibration curve of MS in Methanol at 223 nm

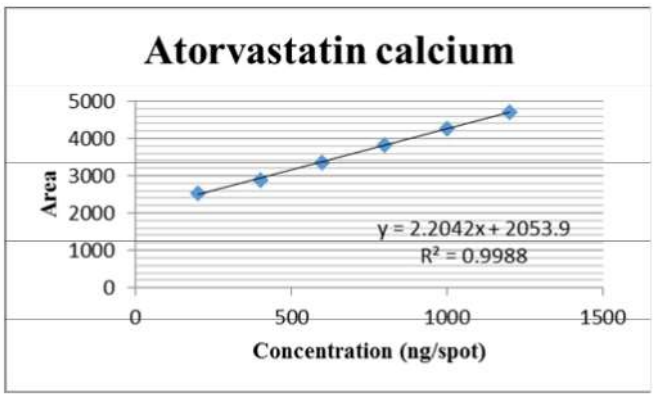


Figure 6: Calibration curve of ATR in Methanol at 223 nm

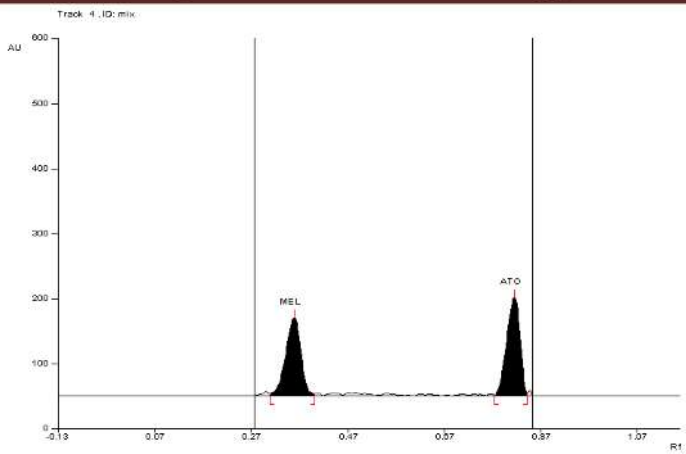


Figure 7: HPTLC Chromatogram of Standard MS and ATR in mixture

RESULT AND DISCUSSION

Method development

The TLC procedure was optimized for simultaneous determination of MS and ATR. The mobile phase Acetonitrile: Methanol: Ethyl acetate: Glacial acetic acid (2: 4: 4: 0.06 % v/v/v/v) resulted in good resolution with sharp and symmetrical peaks of R_f 0.32 ± 0.005 for MS and 0.77 ± 0.004 for ATR. It was observed that pre-washing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured reproducibility and peak shape of both drugs (Figure 3 and 7).

Validation

Linearity

Linear regression data for the calibration plots revealed linear relationships between area and concentration over the ranges 500-3000 ng/spot for MS and 200-1200 ng/spot for ATR. The linear equations for the calibration plots were y = 1.3202x + 26.08 and y = 2.2149x + 2015.5 with correlation coefficient (r) being 0.9973 and 0.9984 for MS and ATR, respectively (Table 1 and 2).

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in (Table 3 and 4) reveal the high precision of the method.

Accuracy

When the method was used for accuracy and subsequent analysis of both drugs from the synthetic mix, and spiked with 50, 100, and 150% of additional drug, the recovery was 99.48- 100.42 % for MS and 99.26- 100.97 % for ATR (Table 5).

Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 0.843261 and 2.5553 ng/spot for MS and 27.8408 and 84.3662 ng/spot for ATR.

Specificity

The peak purity of MS and ATR was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9995 and r (M, E) =0.9994 for MS and r (S, M) = 0.9998 and r (M, E) = 0.9991 for ATR. Good match was obtained between standard and sample spectra of MS and ATR respectively. (Figure 4)

Repeatability

The % RSD for peak area values of MS and ATR was found to be 0.9650 and 0.3806, respectively as given in Table 6.

Analysis of MS and ATR in marketed formulation

When synthetic mixture was analyzed, MS and ATR gave sharp and well defined peaks at R_f 0.32± 0.005 and 0.77± 0.004, respectively, when scanned at 223 nm. The results in Table 7 indicate that there was no interference from the excipients commonly present in the tablets. The % purity was 100.43% for MS and 100.29% for ATR.

CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used in future for simultaneous determination of MS and ATR in tablets as method gave results within range for synthetic mixture. The method was validated as per International Conference on Harmonization (ICH) guidelines.

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