Development and Validation of HPTLC Method for Simultaneous Determination of Amlodipine Besylate and Metoprolol Succinate in Bulk and Tablets

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A simple, selective, precise high-performance thin-layer chromatographic method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and pharmaceutical combined dosage form was developed and validated. The method employed HPTLC aluminum plates precoated with silica gel 60F-254 (10×10) as the stationary phase. The solvent system consisted of toluene:ethyl acetate:methanol:triethylamine (4:1:1:0.4 v/v/v). The system was found to give a compact spot for amlodipine besylate (R_f = 0.39±0.02) and metoprolol succinate (R_f = 0.59±0.02). Densitometric analysis of amlodipine besylate and metoprolol succinate was carried out in the absorbance mode at 254 nm. Linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9990±0.0013 with respect to peak area in the concentration range 400-1400 ng per spot for amlodipine besylate and r^2 = 0.9993±0.0013 with respect to peak area in the concentration range 3800–13300 ng per spot for metoprolol succinate. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 39.99 and 121.20 ng per spot for amlodipine besylate and 234.31 and 710.03 ng per spot for metoprolol succinate, respectively. Statistical analysis proved that the method is selective, precise and accurate for the estimation of amlodipine and metoprolol.

Key words: Amlodipine besylate, HPTLC, metoprolol succinate, pharmaceutical formulation

Amlodipine besylate (AMB, fig. 1), chemically, (RS)-3-ethyl-5-methy-l-2-(2-amino ethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzene sulfonate[1], is a long acting calcium channel blocker, which is used as an antihypertensive agent[2-4]. Metoprolol succinate (MTS, fig. 1), is chemically (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy] propan-2-ol, a selective β_1-receptor blocker, which is also used as an antihypertensive agent[5].

For estimating AMB, methods have been reported using HPLC, HPTLC and UV spectrophotometry alone or in combination with other drugs[6-11]. Various methods have been reported for the analysis of MTS in bulk and in pharmaceutical formulation such as those using HPLC, ultra performance liquid chromatography (UPLC) with different column materials and mobile phase systems[12-16]. This method developed has chosen over the reported HPTLC method owing to a better mobile phase composition of the method reported[17].

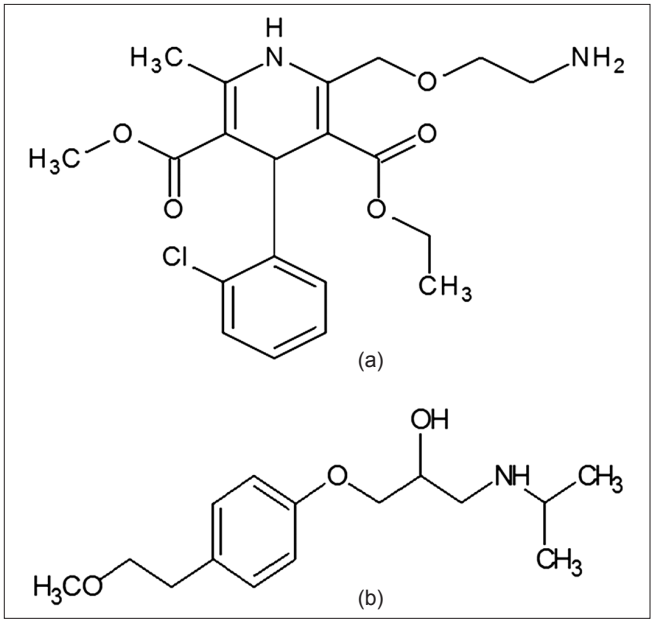

Fig. 1: Chemical structure of analytes.
Chemical structure of of amlodipine besylate (a) and metoprolol succinate (b)

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Literature review revealed that no HPTLC method has been reported for estimation of AMB and MTS as single components or as a mixture. The present study reports development and validation of a simple, accurate, economical and reproducible method for the analysis of AMB and MTS using HPTLC at 254 nm either as bulk drug mixture or in combined tablet dosage form.

MATERIAL AND METHODS

AMB and MTS were provided as gift samples by Sun Pharmaceuticals Ltd., Mumbai, India. Toluene, methanol, ethyl acetate and triethylamine were used as solvents to prepare the mobile phase. All chemicals used were of HPLC grade (S. D. Fine Chem. Ltd., Mumbai, India) used without further purification.

Instrumentation and HPTLC conditions:

The samples were spotted in the form of bands of width 6 mm with 100 µl sample syringe on precoated silica gel aluminium plate 60 F_{254} (10×10 cm, E Merck, Darmstadt, Germany) using a Camag Linomat 5 (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110° for 5 min, prior to chromatography. A constant application rate of 150 nl/sec was employed and space between two bands was maintained at 14 mm. The slit dimension was kept at 6×0.45 mm. The mobile phase consists of toluene:ethyl acetate:methanol:triethylamine (4:1:1:0.4 v/v/v). Linear ascending development was carried out in 10×10 cm twin trough glass chamber. The optimized chamber saturation time for mobile phase was 30 min, at temperature (25±2°) and relative humidity (60±5%); the length of chromatogram run was 8 cm and TLC plates were air- dried. Densitometric scanning was performed on a Camag TLC Scanner 3 equipped with winCATS software version 1.3.0 at 254 nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using peak area with linear regression.

Preparation of standard solution:

An accurately weighed quantity (10 mg) of AMB and 95 mg of MTS were transferred to 10 ml volumetric flask containing 4 ml methanol and volume was adjusted to mark with methanol to obtain a concentration of 1000 ng/µl of AMB and 9500 ng/µl of MTS. Dilutions were prepared from the stock solution of AMB and MTS. For AMB linearity range employed was 400-1400 and for MTS 3800-13300 ng/spot.

Analysis of tablets:

Twenty Metpure AM 2.5 (25 mg MTS + 2.5 mg AMB) tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to 2.5 mg of AMB and 23.75 mg of MTS was transferred to 25 ml volumetric flask, extracted with methanol for 20 min by shaking mechanically. The solution was diluted to volume with the same solvent and filtered. A sample solution of 6 µl was spotted on TLC plate followed by development and scanning as described in instrumentation and HPTLC condition section. The concentration of drugs was determined from linear regression equations and % label claim was calculated. The developed method was validated in terms of linearity, specificity, precision, accuracy, robustness and ruggedness[18].

RESULTS AND DISCUSSION

In this study, quantitative determination of AMB and MTS in tablets was performed by a HPTLC method. The HPTLC developed was found to be simple, rapid and sensitive, which did not require any pretreatment procedure. Typical overlain spectra of AMB and MTS were shown in fig. 2**.** Also the typical HPTLC Chromatogram obtained from the analysis of standard AMB (R_f = 0.39) and MTS (R_f = 0.59) was shown in fig. 3. The peak purity of AMB and MTS were found to be 0.999 and 0.998, respectively indicating that no impurities or degradation products were found along with the peaks of standard drug solutions, hence making the method specific. Regression analysis for the HPTLC method was carried out (Tables 1 and 2) and a highly linear calibration graph

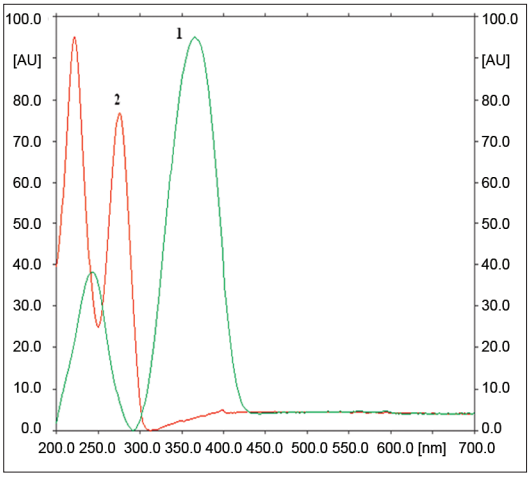

Fig. 2: Overlain spectra of samples.
Typical overlain spectra of AMB (1) and MTS (2) standards AMB represents amlodipine besylate and MTS represents metoprolol succinate.

(r^2 = 0.999) indicated adherence of the method to Beer's law. Quantitative determination of AMB and MTS in tablets using this HPTLC method indicated good agreement with the labeled amount of AMB and MTS (Table 3). Closeness of the amount found to the amount taken and the low coefficient of variation value showed that the proposed method was accurate and precise. Recovery study conducted by the HPTLC method was performed by spiking 80, 100 and 120 % of additional drug recovery of 99.48-100.87% for AMB and 99.85-100.88 as listed in Table 4. The method was found to be precise based on the results obtained in the intra-day and inter-day precision evaluation study. These results were expressed in terms of % RSD that was found to be less than 2 (Table 5). High recovery values followed by low % RSD value (<2) coupled with low standard deviation makes the proposed method highly suitable for accurate and precise determination of AMB and MTS in combined tablet dosage forms. Repeatability was determined by spotting 8 µl of standard solutions on a TLC plate. After developing the plate, separated spots of AMB and MTS were scanned six times without changing position of the plate and RSD for measurement of peak area was calculated, which was found to be 0.9391 and 0.8947% for AMB and MTS, respectively (Table 6).

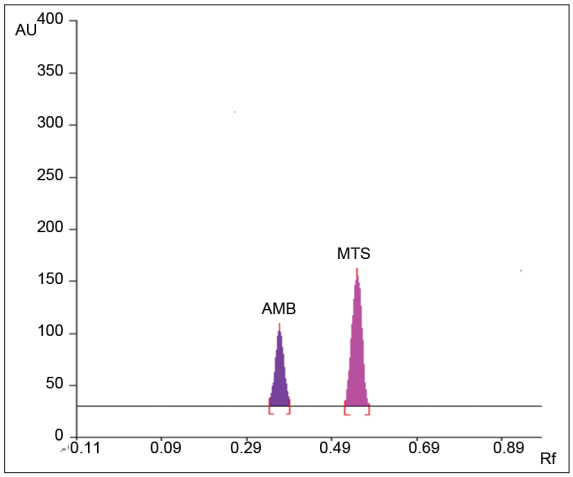

Fig. 3: Typical HPTLC chromatograms of AMB and MTS Typical HPTLC chromatograms of AMB (amlodipine besylate, R_f=0.39) and MTS (metoprolol succinate, R_f=0.59) in mobile phase consisting of toluene:ethyl acetate:methanol:triethylamine (4:1:1:0.4 v/v/v) at 254 nm

TABLE 1: STATISTICAL ANALYSIS FOR THE CALIBRATION CURVES OF AMB AND MTS

Concentration (ng/spot)	Peak area (mean±SD; n=5)	% RSD
AMB		
400	793.94±11.70	1.47
600	1177.48±10.98	0.93
800	1544.76±14.50	0.93
1000	1950.34±23.59	1.20
1200	2267.44±30.43	1.34
1400	2708.42±29.11	1.07
MTS		
3800	1605.52±15.59	0.97
5700	2364.82±25.68	1.08
7600	3022.62±30.69	1.01
9500	3809.96±15.27	0.40
11400	4537.82±29.27	0.64
13300	5182.86±33.37	0.64

AMB represents amlodipine besylate and MTS represents metoprolol succinate

TABLE 2: DETERMINATION OF AMB AND MTS IN TABLET FORMULATION

Drugs	Label claim (mg)	Amount found (mg)	Amount found (%)
AMB	2.5	2.4676	98.70
	2.5	2.5149	100.59
	2.5	2.4935	99.74
	2.5	2.5191	100.76
	2.5	2.5131	100.52
Mean±SD		2.5016±0.0214	100.06±0.8576
% RSD		0.8570	0.8570
MTS	23.75	24.2147	101.9568
	23.75	23.5162	99.0158
	23.75	24.0145	101.1139
	23.75	23.8671	100.4932
	23.75	23.6207	99.4558
Mean±SD		23.7439±0.1742	99.9745±0.7335
% RSD		0.7337	0.7337

Brand name of the tablets used was Metpure-AM 2.5, with Batch No.LAA07008 manufactured by M/s Emcure Ltd., Pune

TABLE 3: RECOVERY ANALYSIS OF AMB AND MTS IN TABLETS

Components	Initial amount (ng/spot)	Amount added (%)	Amount recovered±SD (ng/spot, n=3)	% Recovered	% RSD
AMB	600	0	602.43±6.27	100.40	1.04
	600	80	1074.38±7.08	99.48	1.48
	600	100	1199.52±8.17	99.96	1.36
	600	120	1331.48±6.76	100.87	0.93
MTS	5700	0	5691.86±41.30	99.85	0.72
	5700	80	10249.88±50.03	100.34	1.09
	5700	100	11438.76±42.90	100.24	0.75
	5700	120	12650.32±72.21	100.88	10.4

AMB represents amlodipine besylate and MTS represents metoprolol succinate

TABLE 4: INTRA-DAY AND INTER-DAY PRECISION STUDIES

Drugs	Conc. (ng/spot)	Intra-day amount found (ng)		Inter-day amount found (ng)	
		Mean±SD (n=5)	% RSD	Mean±SD (n=5)	% RSD
AMB	600	604.92±6.02	0.99	601.62±5.05	0.84
	800	801.00±4.59	0.57	797.81±9.39	1.17
	1000	997.57±7.23	0.72	996.81±12.34	1.23
MTS	5700	5613.06±39.72	0.68	5713.33±71.79	1.25
	7600	7610.55±45.91	0.60	7611.95±46.42	0.60
	9500	9495.53±76.11	0.80	9563.82±16.06	0.16

AMB represents amlodipine besylate and MTS represents metoprolol succinate

TABLE 5: RESULTS OF REPEATABILITY STUDIES

Application volume [µl]	Area AMB (800 ng/spot)	Area MTS (7600 ng/spot)
8	1548.6	3016.4
8	1542.8	3057.8
8	1562.6	3069.8
8	1523.3	3088.4
8	1551.5	3071.5
8	1529.8	3029.8
Mean	1543.1	3055.617
SD	14.4919	27.34011
%RSD	0.9391	0.894749

AMB represents amlodipine besylate and MTS represents metoprolol succinate

TABLE 6: RUGGEDNESS STUDIES

Analyst	% Amount found AMB (*n* = 3)	% RSD	% Amount found MTS (*n* = 3)	%RSD
I	100.23	1.38	100.32	0.84
II	100.02	0.78	100.26	0.76

AMB represents amlodipine besylate and MTS represents metoprolol succinate

The results of ruggedness and robustness evaluation were shown in Table 7.

The developed HPTLC technique is found to be precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines. Statistical analysis indicated that the method is repeatable and selective for the analysis of AMB and MTS both in bulk drug mixture and in tablets.

The developed method appears to be useful for determining purity of these drugs available from various sources. It is necessary to evaluate this method further in degradation kinetics of AMB and MTS and also for estimating these drugs in plasma and other biological fluids. In conclusion, the proposed HPTLC method is suitable for the analysis of amlodipine besylate and metoprolol succinate in commercial tablets.

TABLE 7: RESULTS OF ROBUSTNESS STUDIES

Parameters	AMB		MTS	
	SD of peak area (n=6)	% RSD	SD of peak area (n=6)	% RSD
Mobile phase composition Toluene:ethyl acetate: methanol:triethylamine; 4:1:1:0.4 (v/v/v)	16.84	1.09	22.09	0.72
Toluene: ethyl acetate: methanol:triethylamine; 3.5:1.3:1.2:0.4 (v/v/v)	24.80	1.59	31.00	1.01
Mobile phase volume				
6.4 ml	18.56	1.18	18.56	0.60
12.4 ml	21.32	1.36	21.32	0.69
Development distance				
7 cm	13.82	0.89	13.82	0.45
7.5 cm	22.24	1.41	22.24	0.72
8 cm	28.61	1.85	34.71	1.13
Relative humidity				
55	11.09	0.71	11.09	0.36
65	15.85	1.03	18.41	0.60
Duration of saturation				
20 min	14.88	0.95	21.51	0.70
25 min	18.69	1.20	10.94	0.35
30 min	18.88	1.22	22.02	0.71
Activation of prewashed TLC plates				
8 min	14.06	0.90	14.06	0.46
10 min	28.48	1.56	28.58	0.93
12 min	12.78	0.82	29.70	0.96
Time from spotting to chromatography	13.33	0.89	28.77	0.93
Time from chromatography to scanning	12.84	0.84	26.35	0.86

AMB represents amlodipine besylate and MTS represents metoprolol succinate

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