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Development and Validation of TLC-Densitometry Method for Simultaneous Determination of Telmisartan and Amlodipine Besylate in Bulk and Tablets

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ABSTRACT

A rapid, simple, and selective high performance thin layer chromatographic method was developed and validated for simultaneous estimation of telmisartan and amlodipine besylate in pharmaceutical dosage forms. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system comprised: tetrahydrofuran: dichloroethane: methanol: ammonia solution (6.0:2.0:1.0:0.4 v/v). This system was found to give compact spots for both telmisartan (Rf value of 0.22 ± 0.02) and amlodipine besylate (Rf value of 0.45 ± 0.02). Spectrodensitometric scanning-integration was performed at a wavelength of 326 nm. The polynomial regression data for the calibration plots showed good linear relationship with r2 = 0.9993 in the concentration range of 1,200–7,200 ng for telmisartan and 400–1,400 ng for amlodipine besylate with r2 = 0.9996. The method was validated for precision, accuracy, ruggedness, and recovery. The minimum detectable amounts were found to be 149.41 ng and 53.07 ng for telmisartan and amlodipine besylate, respectively. The limits of quantitation were found to be 452.78 ng for telmisartan and 160.83 ng for amlodipine besylate. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of telmisartan and amlodipine besylate.

Key words: Amlodipine besylate, HPTLC, simultaneous determination, telmisartan

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INTRODUCTION

Telmisartan, 4-[{2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl} methyl]-biphenyl-2-carboxylic acid [Figure 1] is a new highly selective, nonpeptide angiotensin II type 1 (AT1)-receptor antagonist.[1] Telmisartan lowers blood pressure through blockade of the rennin-angiotensin-aldosterone system (RAAS) and is widely used in the treatment of hypertension.[2] In the literature, quantitation of telmisartan in urine sample has been widely used.[3] Also, determination of telmisartan in human plasma by liquid chromatography-tandem mass spectrometry has been reported.[4] An RP-HPLC method for determination of telmisartan in combination with hydrochlorothiazide has been reported by Wankhede *et al*.[5] Bhat *et al*.[6] have reported difference spectrophotometric method for determination of telmisartan. Also, Junfeng song *et al*.,[7] reported linear sweep polarographic method for determination of telmisartan.

Amlodipine besylate, chemically, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine-dicarboxylic acid 3-ethyl, 5-methyl ester [Figures 2,3], is an antihypertensive and an antianginal

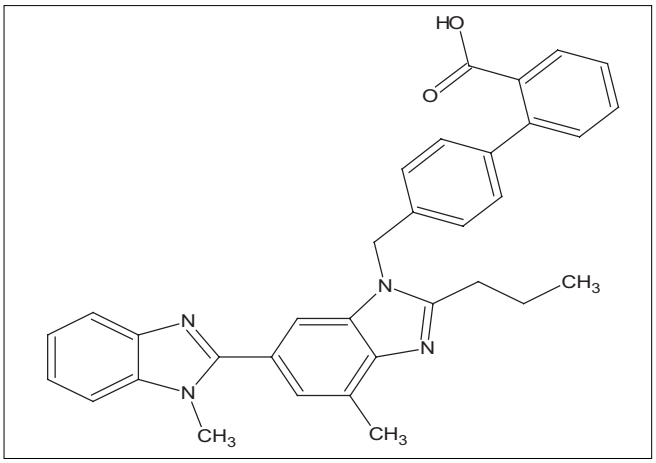

Figure 1: Structure of Telmisartan

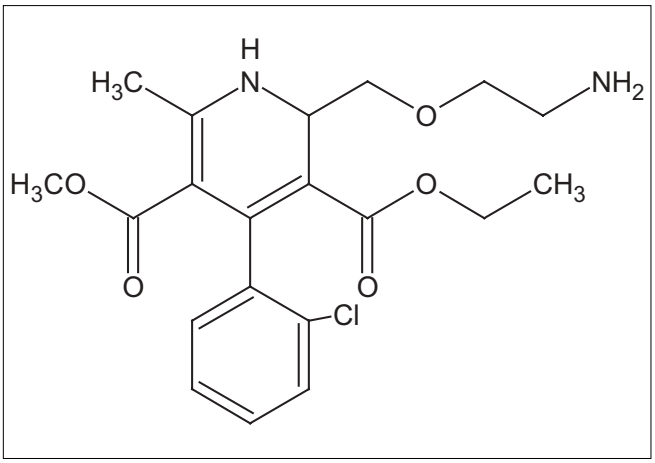

Figure 2: Structure of amlodipine besylate

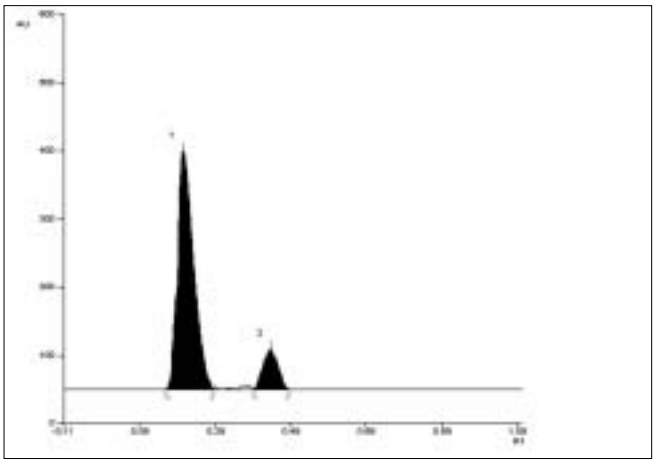

Figure 3: TLC—Densitometric chromatogram of telmisartan (1) and amlodipine besylate (2)

agent in the form of the besylate salt, amlodipine besylate.[1] Amlodipine besylate is official in British pharmacopoeia.[8] Several methods for quantitative estimation of amlodipine besylate in pharmaceutical dosage form and in biological fluids have been reported in the literature. M. Joseffson *et al*.[9] have reported HPLC method for amlodipine besylate in plasma with amperometric detection and a single step solid phase sample perparation. A. Zarghi *et al*.[10] have also reported HPLC method for amlodipine besylate in plasma. A. Ceccato *et al*.[11] reported LC-MS method for determination of amlodipine besylate in human plasma. Several RP-HPLC methods for determination of amlodipine besylate in combination with atorvastatin calcium have been reported.[12–14] D. Jain and M. R. Khan have also reported spectrophotometric method for estimation of amlodipine besylate in combination with atorvastatin calcium.[15]

However, to our knowledge, there is no method for the simultaneous determination of these two drugs by high-performance thin-layer chromatography (HPTLC) in the literature.

The aim of this work is to develop an accurate, specific, repeatable, and validated method for simultaneous determination of telmisartan and amlodipine besylate in both bulk and tablet formulations.

EXPERIMENTAL

Materials

Telmisartan and amlodipine besylate were gift samples from Virdev Intermediates Ltd., India and Cadila Pharmaceuticals Ltd., India, respectively. All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

HPTLC instrumentation

Spotting was done in the form of 6 mm bands with Camag microlitre syringe on precoated silica gel aluminium plate 60 F-254 (20 × 10 cm^2 with 0.2 mm thickness; Merck, Germany) using a Camag Linomat V (Switzerland). The solvent system comprised tetrahydrofuran: dichloroethane: methanol: ammonia solution (3.0:1.0:0.5:0.2 v/v). Chromatogram was developed in a camag twin trough chamber using a linear ascending technique. The chamber saturation time for mobile phase was optimized to 30 min. The length of chromatogram run was approximately 80 mm. Subsequent to the development; the TLC plates were dried in a current of air. The densitometric analysis was performed on a Camag TLC scanner III in the absorbance mode at 326 nm.

Calibration plots

Stock solutions of telmisartan (1 mg/ml) and amlodipine besylate (1 mg/ml) were prepared in methanol. A series of standard curves were prepared over a concentration range of 1,200–7,200 ng for telmisartan. For amlodipine besylate the stock solution was spotted to give concentrations in the range of 400–1,400 ng. The data of spot area versus drug concentration was treated by linear least square regression analysis.

Method validation

Method was validated in compliance with ICH guidelines.[16] The following parameters were validated.

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (4800 ng per spot of telmisartan and 600 ng per spot of amlodipine besylate). The intra and interday variation for the determination of telmisartan and amlodipine besylate was carried out at three different concentration levels of 3,600, 4,800, and 6,000 ng per spot and 450, 600, 750 ng per spot, respectively.

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like tetrahydrofuran: dichloroethane: methanol: ammonia (5.8:2.0:1.2:0.4 *v/v*), tetrahydrofuran: dichloroethane: methanol: ammonia (6.2:2.0:0.8:0.4 *v/v*) were tried and chromatograms were run. The duration of saturation time was varied from 20 mins, 25 mins, and 30 mins. The amount of mobile phase was 4.7 ml and 9.4 ml, development distance was varied from 70, 75, and 80 mm, respectively. The relative humidity was 55 % and 65 %. The plates were prewashed by methanol and activated at 60 °C ± 5 for 8, 10, and 12 mins, respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was 2 hrs. Robustness of the method was done at concentration of 4800 ng per spot for telmisartan and 600 ng per spot for amlodipine besylate.

Limit of detection and limit of quantification

In order to determine detection and quantification limit, drugs concentrations in the lower part of the linear range of the calibration curves were used. Stock solutions of 1,000 μg/ml were prepared for both drugs and different volume of stock solution 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 μl for telmisartan and 0.40, 0.45, 0.50, 0.55, 0.60, 0.65 μl for amlodipine besylate were spotted in triplicate. The amount of both drugs by spot versus average response (peak area) was graphed and the equations for this were determined. The standard deviations (SD) of responses were calculated. The average of standard deviations was calculated (ASD). Detection limit was calculated by $(3.3\times ASD)/b$ and quantification limit was calculated by $(10\times ASD)/b$, where "*b*" corresponds to the slopes obtained in the linearity study of method for both drugs.

Specificity

The specificity of the method was ascertained by analyzing standard drugs and samples. The spot for both drugs in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of both drugs was accessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions of the spot.

Recovery studies

The analysed sample was over spotted with extra 80, 100 and 120 % of the standard drugs and it was analysed by the proposed method. At each level of the amount, three determinations were performed. This was done to check the recovery of the drug at different level in the formulation.

Analysis of pharmaceutical formulation

To determine the content of both the drugs from the tablet formulation (label claim: 40 mg/tablet of telmisartan and 5 mg/tablet of amlodipine besylate), 20 tablets were powdered and powder equivalent to 40 mg of telmisartan and 5 mg of amlodipine besylate was weighed. Methanol was used for extraction. To ensure complete extraction of the drug it was sonicated for 15 mins and the solution was made up to 50 ml. Solution (6 μl) was spotted onto the plate followed by development and scanning. The analysis was repeated in six replicates. The possibility of excipient interference in the analysis was studied.

RESULTS AND DISCUSSION

Standardization of chromatographic conditions

Various solvent systems were evaluated to arrive at an optimum resolution of both drugs. The solvent system comprised tetrahydrofuran: dichloroethane: methanol: ammonia (6.0:2.0:1.0:0.4 *v/v*) gave dense, compact and well separated spots of the drugs from the mixture. The R_f values were found to be 0.22 and 0.45 for telmisartan and amlodipine besylate, respectively. Densitometric analysis of telmisartan and amlodipine besylate was performed at

326 nm. Adequate separation of the two drugs enabled the development of a selective and specific method of analysis.

Standard curves

The polynomial regression data for the calibration plots (n = 6) showed a good linear relationship over a concentration range of 1,200–7,200 ng for telmisartan and 400–1,400 ng for amlodipine besylate. The mean values of intercept, slope and correlation coefficient are shown in Table 1.

Method validation

Precision

The repeatability of sample application and measurement of peak area were expressed in the terms of percentage RSD and results are depicted in Table 2, which revealed intra and interday variation of telmisartan and amlodipine besylate at three different concentration levels 3,600, 4,800, 6,000 ng per spot and 450, 600, 750 ng per spot, respectively.

Robustness of the method

The standard deviation of peak areas was calculated for parameter and %RSD was found to be less than 2%. The low value of %RSD value as shown in Table 3 indicated robustness of the method.

LOD and LOQ

Detection limit and quantification limit were calculated and found 149.41 and 452.78 ng for telmisartan and 53.07 and 160.83 ng for amlodipine besylate, respectively. This indicates the adequate sensitivity of the method.

Specificity

The peak purity of telmisartan and amlodipine were assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot.

Recovery studies

The proposed method when used for extraction and subsequent estimation of both drugs from pharmaceutical dosage form after spiking with 80, 100, and 120% of additional drug afforded recovery of 99–101% as listed in Table 4.

The summery of validation parameters were listed in Table 5.

Analysis of pharmaceutical formulation

The spots at R_f 0.22 (for telmisartan) and 0.45 (for amlodipine besylate) were observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 101.11% ± 0.81 (%RSD of 0.80) and 100.33% ± 0.85 (%RSD of 0.84) for

Table 1: Linear regression data for the calibration curve

Parameters	Telmisartan	Amlodipine besylate
Linearity range (ng per spot)	1,200–7,200	400–1400
r^2 ± SD	0.9993 ± 0.0004	0.9996 ± 0.0002
Slope ± SD	0.8525 ± 0.01	2.1015 ± 0.01
Intercept ± SD	6,543.6 ± 64.97	789.49 ± 20.28

Table 2: Intraday and interday precision

Drugs	Amount (ng per spot)	Amount found (ng per spot)	SD	%RSD
Intraday precision				
Telmisartan	3,600	3,594.25	18.88	0.52
	4,800	4,798.24	10.90	0.22
	6,000	6,048.05	12.19	0.20
Amlodipine besylate	450	450.31	2.64	0.58
	600	603.41	4.35	0.72
	750	749.34	3.35	0.44
Interday precision				
Telmisartan	3,600	3,630.81	21.77	0.59
	4,800	4,756.26	8.83	0.18
	6,000	6,119.88	17.30	0.28
Amlodipine besylate	450	457.89	3.73	0.81
	600	601.81	7.22	1.20
	750	755.43	3.73	0.49

Table 3: Robustness of the method

Parameters	Telmisartan		Amlodipine besylate	
	SD of peak area	% RSD	SD of peak area	% RSD
Mobile phase composition				
A	23.89	0.22	9.91	0.48
B	15.41	0.14	7.26	0.35
Mobile phase volume				
4.7 ml	15.38	0.14	9.99	0.48
9.4 ml	11.37	0.10	9.47	0.46
Development distance				
70 mm	18.84	0.39	2.47	0.41
75 mm	08.05	0.16	2.34	0.39
80 mm	13.85	0.29	4.05	0.67
Relative humidity				
55%	16.73	0.15	11.73	0.57
65%	13.80	0.12	10.95	0.53
Duration of saturation				
20 min	19.31	0.18	6.08	0.29
25 min	17.10	0.16	9.12	0.44
30 min	12.22	0.11	6.54	0.31
Activation of prewashed TLC Plates				
08 min	12.66	0.11	5.46	0.26
10 min	5.22	0.04	5.93	0.28
12 min	7.19	0.06	5.58	0.27
Time from spotting to chromatography	7.22	0.06	5.15	0.25
Time from chromatography to scanning	11.18	0.10	5.34	0.25

a = tetrahydrofuran: dichloroethane: methanol: ammonia (5.8:2.0:1.2:0.4 *v/v*);
b = tetrahydrofuran: dichloroethane: methanol: ammonia (6.2:2.0:0.8:0.4 *v/v*)

Table 4: Recovery studies

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	% RSD
(a) Telmisartan			
80	3840	100.77	0.83
100	4800	100.36	0.18
120	5760	100.69	0.44
(b) Amlodipine besylate			
80	480	100.99	0.17
100	600	101.52	0.35
120	720	100.73	0.29

Table 5: Summary of validation parameters

Parameters	Telmisartan	Amlodipine besylate
Linearity range (ng per spot)	1,200–7,200	400–1,400
Correlation coefficient	0.9993	0.9996
Limit of detection (ng per spot)	149.41	53.07
Limit of quantitation (ng per spot)	452.78	160.83
Recovery (n = 9)	100.60 ± 0.21	101.08 ± 0.40
Precision (%RSD)		
Repeatability	0.79	0.52
Intraday	0.20–0.52	0.44–0.72
Interday	0.18–0.59	0.49–1.20
Ruggedness (%RSD)		
Analyst-I (n = 6)	0.48	0.76
Analyst-II (n = 6)	0.37	0.56
Robustness	Robust	Robust
Specificity	Specific	Specific

telmisartan and amlodipine besylate, respectively. It may therefore be inferred that degradation of telmisartan and amlodipine besylate had not occurred in the marketed formulations that were analyzed by this method. The low %RSD value indicated the suitability of this method for routine analysis of telmisartan and amlodipine besylate in pharmaceutical dosage form.

CONCLUSION

The developed HPTLC technique is precise, specific, accurate and robust for the analysis of telmisartan and amlodipine besylate in tablets without the interference of any excipients. The statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of telmisartan and amlodipine besylate as a bulk drug solution and in pharmaceutical formulations.

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