Research Article

Normal and Reversed-Phase HPTLC Methods for Simultaneous Estimation of Telmisartan and Metoprolol Succinate in Pharmaceutical Formulation

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Received 23 August 2011; Accepted 13 September 2011

Academic Editors: T. Bolanca, R. K. Jyothi, T. Macko, and W. Misiuk

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Two simple precise normal-phase (Method I) and reversed-phase (Method II) HPTLC/densitometry method have been developed for simultaneous determination of telmisartan and metoprolol succinate in bulk and in combined tablet formulation. Method I was developed with aluminium plates precoated with silica gel 60F₂₅₄S, and toluene : propanol : methanol : triethylamine (8 : 1 : 1 : 0.5 v/v) was used as mobile phase. Method II was carried out using aluminium coated with RP-18 silica gel 60 F₂₅₄S HPTLC plates using methanol : water : triethylamine (6 : 4 : 0.5 v/v) as mobile phase. Both analyses were scanned with a densitometer at 242 nm. In Method I, good separation and resolution of drugs were achieved with R_f values 0.45 ± 0.02 (telmisartan) and 0.70 ± 0.02 (metoprolol), while in Method II, telmisartan and metoprolol showed R_f values 0.55 ± 0.02 and 0.41 ± 0.02 , respectively. These methods can be used in routine pharmaceutical analysis.

1. Introduction

Telmisartan (TELM), 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1H benzimidazole]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid, is an angiotensin II antagonist used as antihypertensive agent [1]. TELMI blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT₁ receptor in many tissues, such as vascular smooth muscle and the adrenal gland [2, 3]. TELMI has been estimated by various methods like spectrophotometric [4], HPLC [5, 6], HPTLC [7–10], and LC-MS [11, 12] in pharmaceutical formulations and biological fluids.

Metoprolol succinate (MET), Bis[(2RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] butanedioate, is a cardioselective beta blocker used in the management of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, and heart failure [13]. MET has been estimated by spectrophotometric [14], HPLC [15–20], and HPTLC [21, 22] methods.

However, there have been no reports concerning the simultaneous determination of TELMI and MET.

In the present work, an effort has been made to estimate TELMI and MET in bulk and in combined tablet dosage form by NP-HPTLC (Method I) and RP-HPTLC (Method II) methods and validated according to ICH guidelines [23].

2. Experimental

2.1. Materials and Reagents. Telmisartan and metoprolol were provided as a gift sample by Glenmark pharmaceutical Ltd. Nasik. Drugs were used without any further purification. All other reagents required for experimentation were of analytical reagent (AR) grade. For analysis, toluene, methanol, propanol, and triethylamine were purchased from Merck India.

2.2. Instrumentation and Chromatographic Condition. In Method I, chromatography was performed on 20 cm × 10 cm

TABLE 1: Analysis of tablet formulation.

Method	Drug	Label claim (mg)	% Amount found	% RSD ($n = 5$)
I	TELM	40	99.18	0.62
	MET	50	99.69	0.96
II	TELM	40	98.55	0.88
	MET	50	99.12	0.64

aluminium-backed HPTLC plates coated with 200- μm layers of silica gel 60 F₂₅₄S. In Method II, 20 cm $\times$ 10 cm aluminium-backed RP-HPTLC plates coated with 200- μm layers of silica gel 60 RP-18 F₂₅₄S were used. The plates were washed with methanol before chromatographic measurements and activated at 105°C for 5 min. The samples were applied as 6 mm wide bands with the help of Linomat 5 sample applicator (Muttentz, Switzerland) fitted with a 100- μL sample syringe (Hamilton, Bonaduz, Switzerland). The plate was developed in a presaturated Camag twin trough glass chamber (20 cm $\times$ 10 cm). In Method I and Method II, toluene:propanol:methanol:triethylamine (8:1:1:0.5 v/v) and methanol:water:triethylamine (6:4:0.5 v/v), respectively, were used as mobile phases. For Method I and Method II, optimized chamber saturation time was 15 min and 30 min, respectively. The plates were developed to a distance of 8.0 cm and scanned densitometrically using Camag TLC Scanner 3 equipped with winCATS software version 1.3.0 at 242 nm for both methods. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Evaluation was performed using peak area with linear regression.

2.3. Preparation of Standard and Sample Solutions. Stock standard solution of 1 mg mL⁻¹ of TELMI and 2 mg mL⁻¹ were separately prepared.

2.4. Application of the Proposed Method for Simultaneous Estimation of Both the Drugs in Tablets. Twenty tablets (TELMAXX) were weighed; average weight was determined and then crushed in to fine powder. An accurately weighed tablet powder equivalent to 40 mg of TELMI and 50 mg of MET were transferred to 50 mL volumetric flask containing 25 mL methanol and sonicated for 10 min, and volume was adjusted to mark and filtered using 0.45 μm filter (Mill filter, Milford, Mass, USA) and 4 mL of filtrate was further diluted to 10 mL with methanol. Appropriate volume 4 μL was spotted for assay of TELMI and MET. The plates were developed and scanned as described in the above chromatographic conditions in both Method I and Method II (Table 1).

2.5. Preparation of Calibration Curves. From each stock standard solution, 2–8 mL was transferred into two sets of series of seven 10 mL volumetric flasks, and volume was made up to the mark with methanol. From each volumetric flask, a volume 4 μL was applied on HPTLC plate to obtain series of concentration 800–3200 ng per band of TELMI and 1600–6400 ng per band of MET. The plates were developed and

FIGURE 1: Densitogram of TELMI standard (R_f 0.45 $\pm$ 0.02) and MET standard (R_f 0.70 $\pm$ 0.02) measured at 242 nm.

scanned as described under above established chromatographic conditions for both methods. Each standard in six replicates was analyzed, and peak areas were recorded. Calibration curves of TELMI and MET were plotted separately of peak area versus respective concentration of TELMI and MET.

3. Results and Discussion

3.1. Optimization of HPTLC Method

3.1.1. Method I. Optimization of mobile phase was done with a view to separate both these drugs. Various proportions of methanol and toluene were tried as mobile phase. When toluene: *n*-propanol: methanol (8:1:1 v/v) was used as mobile phase, both these drugs were resolved from each other, but the tailing was observed. Therefore, to whip the problem triethylamine was added which has improved the spot characteristic. Finally, toluene: propanol: methanol: triethylamine (8:1:1:0.5 v/v) showed well-defined and resolved peaks when the chamber was saturated with mobile phase for 15 min at room temperature. Both the peaks were well resolved, and no telling observed when plate was scanned at 242 nm. The R_f for TELMI and MET were found to be 0.45 $\pm$ 0.02 and 0.70 $\pm$ 0.02, respectively (Figure 1).

3.1.2. Method II. Firstly, single solvents were selected on the basis of their polarity to separate the spots. Then, the mixtures of solvents were used for separation purpose of TELMI and MET. The spots were developed in mixtures of methanol and water in the ratio of 3:2 v/v. The R_f value obtained was good, but slight tailing was observed. Hence, to reduce the tailing, triethylamine was added in

TABLE 2: Recovery studies.

Method	Drugs	Initial amount (ng <i>per band</i>)	Amount added (%)	% recovery	% RSD ($n = 3$)
Method I	TELMI	1400	80	99.39	0.65
		1400	100	99.59	0.38
		1400	120	99.66	0.28
	MET	1750	80	99.27	0.66
		1750	100	99.01	0.46
		1750	120	99.00	0.52
Method II	TELMI	1400	80	98.83	1.09
		1400	100	99.79	0.78
		1400	120	99.57	0.39
	MET	1750	80	98.27	1.08
		1750	100	99.00	0.61
		1750	120	99.00	0.65

FIGURE 2: Densitogram of MET standard (R_f 0.41 ± 0.02) and TELMI (R_f 0.55 ± 0.02) measured at 242 nm.

the solvent system. Thus, the final mobile phase consisted of methanol : toluene : triethylamine in the ratio (6 : 4 : 0.5 v/v). The chamber saturation time was 30 min. The R_f for TELMI and MET were found to be 0.55 ± 0.02 and 0.41 ± 0.02 , respectively (Figure 2).

3.2. Validation. The method was validated by establishing linearity, accuracy, interday and intraday precision of measurement of sample application. The limit of detection and limit of quantification were also determined.

3.3. Linearity. Linearity was studied in the concentration range from 800–3200 ng *per band* for TELMI and 1600–6400 ng *per band* for MET for both methods. The drugs showed good linearity in the tested range. The regression

coefficient values for TELMI and MET were found to be $r^2 > 0.99$ in both Method I and Method II.

3.4. Accuracy. The accuracy of the experiment was established by spiking preanalyzed sample with known amounts of the corresponding drugs at three different concentration levels, that is, 80%, 100%, and 120% of the drug in the tablet. The spiked samples were then analyzed for three times. The mean recovery is within acceptable limits, indicating both methods are accurate (Table 2).

3.5. Specificity

3.5.1. Method I. The specificity of the method was ascertained by analyzing drug standards and sample. The mobile phase resolved both the drugs very efficiently. The R_f value of TELMI and MET was found to be 0.45 and 0.70, respectively. The peak purity of TELMI extracted from tablet and TELMI standard was tested at the peak-start (S), peak-apex (A), and at the peak-end (E) position (Figure 3). The peak purity of MET extracted from tablet and MET standard was tested by correlating the spectra's of MET at the peak-start (S), peak-apex (A), and at the peak-end (E) positions (Figure 4).

3.5.2. Method II. The mobile phase designed for the method resolved both the drugs very efficiently. The R_f value of TELMI and MET was found to be 0.55 and 0.41, respectively. The peak purity of TELMI extracted from tablet and TELMI standard was tested at the peak-start (S), peak-apex (A), and at the peak-end (E) positions (Figure 5). The peak purity of MET extracted from tablet and MET standard was tested by correlating the spectra's at the peak-start (S), peak-apex (A), and the peak-end (E) positions (Figure 6).

3.6. Ruggedness and Robustness. Ruggedness of the both method was performed for TELMI and MET by two different analysts maintaining similar experimental and environmental conditions.

TABLE 3: Validation parameters.

Method	Method I		Method II	
Parameters	TELMI	MET	TELMI	MET
Linearity (correlation coefficient)	0.997	0.997	0.996	0.998
Slope	3.443	0.381	0.827	0.660
Intercept	2589	732.1	803.5	1135
Ruggedness (% RSD)				
Analyst-I ($n = 6$)	0.71	1.52	0.63	0.90
Analyst-II ($n = 6$)	0.60	0.96	0.87	1.14
Robustness [% RSD] ($n = 6$)				
Mobile phase composition	0.41	0.69	0.87	0.29
Duration of saturation time	0.84	1.23	0.74	0.83
Mobile phase volume	1.49	1.05	0.43	0.43
Development distance	0.38	0.74	0.19	0.65
Sensitivity				
Limit of detection (ng)	2.79	58.69	43.97	64.18
Limit of quantitation (ng)	8.45	177.86	133.26	194.49
Precision (% RSD)				
Intraday ($n = 3$)	0.44–0.73	0.62–0.97	0.51–0.67	0.51–1.13
Interday ($n = 3$)	0.52–0.89	0.46–1.38	0.56–0.79	0.57–1.23
Repeatability ($n = 6$)	0.75	1.40	1.02	0.86

FIGURE 3: Peak purity spectra of standard 1 TELMI, sample 2 extracted from telmisartan-metoprolol tablet, scanned at the peak-start, peak-apex, and peak-end positions of the band (correlation > 0.99).

FIGURE 4: Peak purity spectra of MET standard 1, sample 2 extracted from telmisartan-metoprolol tablet, scanned at the peak-start, peak-apex, and peak-end positions of the band (correlation > 0.99).

Robustness of the method was performed by introducing various changes in the previous chromatographic conditions; effects on the results were examined for both method.

3.7. Sensitivity. The sensitivity of measurements of TELMI and MET by the use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the lowest concentration detected under the chromatographic conditions as the limit of detection (LOD).

LOQ and LOD were calculated by the use equation $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$, where “ N ” is standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of noise, and “ B ” is the slope of

the corresponding calibration curve. The results are recorded for both the methods.

Different validation parameters for the both methods for determining TELMI and MET content were summarized in Table 3.

4. Analysis of Tablet Formulation

In Method I, compact spots of TELMI and MET were obtained with R_f values 0.45 and 0.70, respectively. While in Method II, TELMI and MET were well-resolved chromatogram with R_f values 0.55 and 0.41, respectively. In both these methods, good separation and well-resolved spots were

FIGURE 5: Peak purity spectra of TELMI standard 1, sample 2 extracted from telmisartan-metoprolol tablet, scanned at the peak-start, peak-apex, and peak-end positions of the band (Correlation > 0.99).

FIGURE 6: Peak purity spectra of MET standard 1, sample 2 extracted from telmisartan-metoprolol tablet, scanned at the peak-start, peak-apex, and peak-end positions of the band (Correlation > 0.99).

obtained, which indicates that there is no interferences commonly present in the tablet formulation.

5. Conclusion

The proposed Method I and Method II provide simple, accurate, and reproducible quantitative analysis for simultaneous determination of TELMI and MET in combined tablet dosage form. Both these method were validated according to ICH guidelines.

Acknowledgment

The authors are thankful to R. C. Patel Institute of Pharmaceutical Education and Research.

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