Research Article

SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND TELMISARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC - PDA METHOD

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ABSTRACT

A simple, specific, and accurate reverse phase liquid chromatographic method compatible to mass spectroscopic detection was developed for the estimation of Metoprolol Succinate (MET) and Telmisartan (TEL) in bulk and pharmaceutical dosage forms. A C_{18} reverse phase column (Phenomenex- RP Aqueous) of 250 x 4.6mm, 5 µm particle size with mobile phase containing 15mM ammonium acetate (pH 6.5): acetonitrile (58:42) was used at isocratic mode and eluents were monitored at 230 nm. The retention times of Metoprolol Succinate and Telmisartan were 3.9min and 5.3 min respectively and showed a good linearity in the concentration range of 5-25 µg/mL for Metoprolol Succinate and 2-10µg/mL for Telmisartan with a correlation coefficient (R) of 0.9999 and 0.9999. The percentage assays for bilayered tablets (METOSARTAN) and matrix tablets (TELMAXX) were found to be 99.30, 101.25 and 100.73, 99.56 respectively for Metoprolol Succinate and Telmisartan. The proposed method was validated as per ICH guidelines and successfully applied to the simultaneous estimation of Metoprolol Succinate and Telmisartan in tablet formulations.

Keywords: Metoprolol Succinate, Telmisartan, Simultaneous estimation, RP-HPLC, Validation.

INTRODUCTION

Metoprolol Succinate (MET), chemically is 1-[4-(2-Methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-2-propanol, is a cardio selective β1-adrenergic blocking agent. Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure[1-3]. Telmisartan (TEL), chemically is 4-[(1, 4-dimethyl-2-propyl (2, 6-bi-1H-benzimidazol]-1-yl) methyl][1,1-biphenyl]-2-carboxylic acid, is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance[2-4].

Literature survey revealed HPLC[5-8], LC-MS[9], HPTLC[10,11], and simultaneous UV spectrophotometric methods[12-14] have been reported for the estimation of MET either alone or in combination with other drugs like TEL, Amlodipine, and Olmesartan etc. One analytical method was reported for determination of Tel by VISIBLE spectrophotometric method[15]. Several HPLC methods are also reported for the estimation of MET either alone or in combination either with TEL or with Atorvastatin and Hydrochlorothiazide in different dosage forms[16,17] and degradation product analysis by HPLC[18,19], HPTLC[20] and UPLC[21] were reported in biological samples and pharmaceutical dosage forms. However, two HPLC-UV/PDA methods were published so far for the simultaneous estimation of MET and TEL, in which one method used Carbamazepine as an internal standard and the injection volume used is 50µl and in other method the retention times of MET and TEL were 3.71min and 10.02min (with long retention time for TEL)[5,6]. Based on the linearity range of the drugs in these two methods (MET: 20-30µg/mL, 29.88-69.72µg/mL; TEL: 16-24µg/mL, 24.12-56.27µg/mL), the present investigation is aimed to develop a more sensitive method with low injection volume and also mobile phase compatible to MS detection (the reported methods used phosphate salt buffer which is incompatible with mass spectrophotometric detection). Hence, the present investigation was aimed at developing a simple, rapid, sensitive and economic LC-MS compatible RP-HPLC-PDA method for estimation of MET and TEL which is accurate and precise.

MATERIALS AND METHODS

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex- RP column (250 × 4.6mm, 5µ).

Chemicals and Reagents

MET and TEL were a gift samples from, Aurobindo Pharma Ltd, Hyderabad, India. Acetonitrile, water and ammonium acetate were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade. TELMAXX® (Batch # WDAD06, manufactured

by Glenmark Pharmaceuticals Ltd, Mumbai) is a tablet containing MET (50 mg) and TEL (40 mg), METOSARTAN® (Batch #GKK1423A, manufactured by Sun Pharma Industries Ltd, Jammu & Kashmir) were commercially purchased.

Chromatographic Conditions

Mobile phase consisting of 15mM ammonium acetate (pH 6.5): acetonitrile (58:42) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min in ultrasonic bath before use. The flow rate was 1 mL/min and the injection volume was 20 µL. PDA detection was performed at 230 nm and the separation was achieved at ambient temperature.

Preparation of stock and standard solutions

The stock solution of MET and TEL strength 1mg/mL were prepared by dissolving 10 mg of each drug together in 10mL of methanol in a volumetric flask. Appropriate volumes of these stock solutions were then further diluted with ammonium acetate (diluent) to get the required concentrations of standard solutions at a concentration range of 5-25 µg/mL and 2-10µg/mL.

RESULTS AND DISCUSSION

The present investigation was carried out with a view to develop a RP- HPLC-PDA method for the simultaneous estimation of MET and TEL in bulk and tablet dosage forms. In the present investigation, different mobile phase combinations were tested to develop a highly sensitive LC method, for the analysis of MET and TEL in bulk and formulations. Initial trials were carried with Phenomenex C_{18} column (250 x 4.6 mm) using 15mM ammonium acetate (pH 6.5) and acetonitrile as mobile phase (60:40) with 1.0mL/min using acetonitrile as diluent. MET and TEL were eluted at 4.0min and 6.2min but the peaks were broad at 1 mL/min flow rate. In the next trail flow rate was changed to 1.2mL/min, MET and TEL were eluted at 3.3min and 5.2min, even then the peaks were broad. In other trials the mobile phase composition was changed using 15mM ammonium acetate (pH 6.5) and acetonitrile (58:42) with 1.2mL/min using acetonitrile as diluent, the peaks are eluted at 3.2min and 4.5min but the peaks were broad. Further trials were continued by changing the diluent. 15mM Ammonium acetate was used as the diluent in order to achieve proper peak shape for the 2 peaks. With a mobile phase composition of 15mM ammonium acetate (pH 6.5): acetonitrile (58:42 v/v) at a flow rate of 1 mL/min showed a good resolution, peak shape and desired elution time was obtained and peaks were symmetrical and tailing factor was within the limits and all the peaks were eluted within 10 min run time. The retention times were 3.9, 5.3min respectively for MET and TEL. For quantitative analytical purpose wavelength was set at 230 nm, which provided better reproducibility with minimum or no interference. The method was validated as per ICH guidelines. A sample chromatogram of all 2 standards along with diluent was shown in figure 1. The peak purity indices were also found to be greater than 0.9999 and this indicating peak purity of the all two drug samples used in the analysis and shown in figure 1 along with UV spectra.

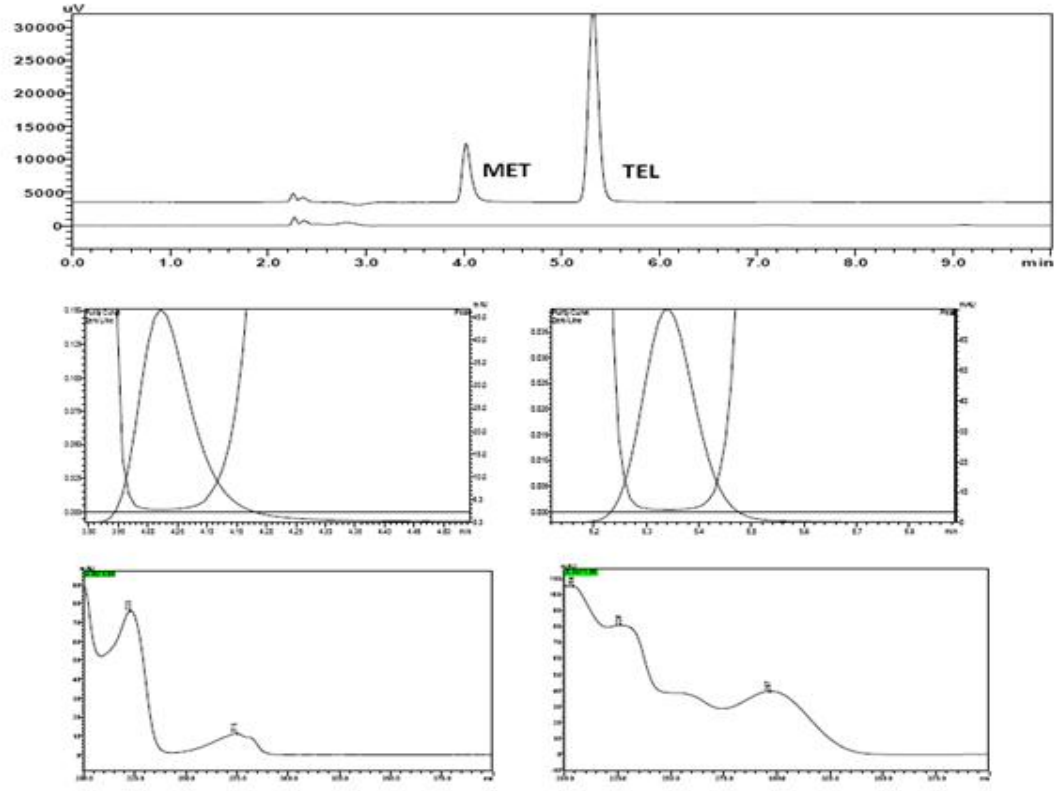

Figure 1: Diluent and MET and TEL chromatograms with Peak purity index and UV spectra

Table 1: Linearity, Accuracy, Precision and Assay data for MET and TEL

	Parameters	MET	TEL
Linearity (n=3)	**Concentration** **Regression equation** **Regression Coefficient (R^2)** **Correlation Coefficient(R)**	5-25ug/mL y =18533x-22739 R^2 = 0.994 R = 0.999	2-10ug/mL y =81555x-77783 R^2 = 0.991 R = 0.999
Accuracy (n=3)	**Level of Addition**	**Mean Percent Recovery (%RSD)**	**Mean Percent Recovery (%RSD)**
	80 %	99.91(0.20)	99.18(0.99)
	100%	101.53(0.35)	100.00(0.57)
	120%	99.71(0.74)	99.91(0.76)
Precision (n=6)			
System Precision	**Average peak area of the standard sample (%RSD)**	70121.33(0.111)	412381.2(0.6)
Method Precision	**Average peak area of the assay sample (%RSD)**	160440.7(0.5)	732984.2(0.1)

Table 2: Robustness data for MET and TEL

Chromatographic parameters	Retention time (min)	Theoretical plates	Capacity factor	Tailing factor	% Assay
MET					
Flow Rate (mL/min)					
0.8	5.02	8208.976	2.790	1.705	101.12
1.00	4.05	7176.278	2.790	1.654	99.90
1.2	3.41	6409.703	2.752	1.642	98.35
Wavelength					
229(-1)	4.05	7177.529	2.790	1.654	101.91
230	4.05	7177.529	2.790	1.654	99.90
231(+1)	4.05	7180.032	2.790	1.654	99.16
TEL					
Flow Rate (mL/min)					
0.8	6.61	11963.650	2.356	1.228	102.36
1.0	5.28	9965.688	2.334	1.207	100.37
1.2	4.27	8409.666	2.198	1.218	99.75
Wavelength					
229(-1)	5.28	9965.625	2.334	1.207	101.29
230	5.28	9965.688	2.334	1.207	100.37
231(+1)	5.28	9964.118	2.334	1.207	99.15

Linearity

A linear relationship was evaluated across a concentration range (5-25 µg/mL) for MET and (2-10µg/mL) for TEL of the analytical procedure in triplicate. The regression data was mentioned in table 1 and showed good linearity in this range.

System Precision

Precision studies were carried out in terms of repeatability. Six determinations of 100 % concentration at 5µg/mL and 4µg/mL level was evaluated and the data given in table 1. The % RSD was found to be below 2 and fulfilled the ICH guidelines criteria.

Method Precision

The method precision was determined by preparing a sample solution of single batch MET and TEL tablet and analyzing as per the proposed method. Repeatability was carried out using six replicates of the same concentration (5µg/mL, 4µg/mL). The data was given in table 1. The % RSD was found to be below 2 and fulfilled the ICH guidelines criteria.

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product. Accuracy was tested by analyzing samples at least in triplicate, at levels of 80,100 and 120% of label claim. These results indicate a good accuracy of the method to that of the labelled claim. The obtained recovery results were given in table 1.

Robustness

Robustness was studied by making deliberate changes in the flow rate and wavelength to evaluate the impact on the method. Retention times were significantly changed with flow rate but no change was found due to change in wavelength, however % assay values, tailing factor, capacity factor and theoretical plate number were within

limits and these results indicated minor changes in the flow rate and wavelength didn't affect the assay results. The data was given in table 2.

LOD & LOQ

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve. LOD for MET and TEL was found to be 0.069, 0.011µg/mL respectively. LOQ for MET and TEL was found to be 0.211, 0.035 µg/mL respectively. These results indicate that the method is sensitive enough to carry out the routine analysis of MET and TEL combination dosage forms.

System Suitability

System suitability studies were carried out by injecting a concentration of 15µg/mL and 6µg/mL standard of MET and TEL at different injection volumes from 10-50µg/mL. The %RSD values for retention times and tailing factor were 1.14, 0.08 and 0.98, 0 respectively.

Specificity

The specificity of the method was established by spiking diluent solution of commonly used excipients in the form of tablet and showed no peaks within the retention time of two drugs and also over the range of 10.0min as shown in figure 2 & 3.

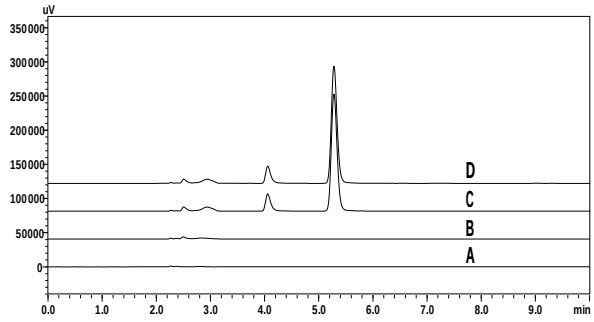

Figure 2: Chromatograms of A: Placebo, B: Blank, C: Sample, D: Standard samples

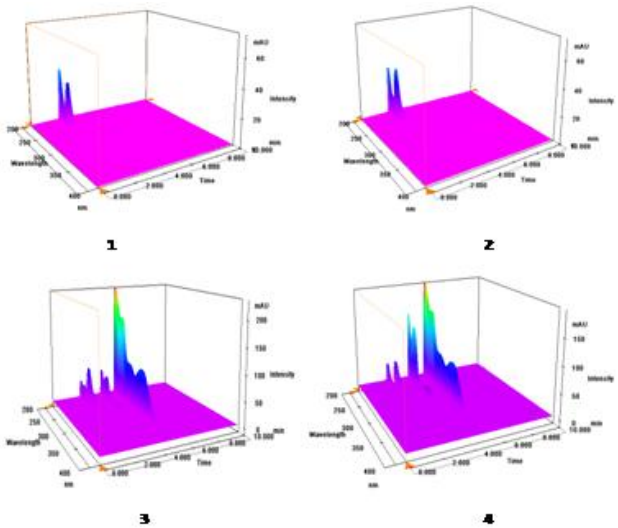

Figure 3: 3 D Plots of diluent (1), Placebo (2), Sample (3), Standard chromatograms (4)

Assay

The amount present in the each tablet was calculated by comparing the area of standard with that of tablet sample. The percentage content of MET and TEL was found to be 99.30 and 101.25 for METOSARTAN tablets and 100.73 and 99.56 for TELMAXX tablets respectively. The assay was found to be within the limits and the present LC conditions can be used for the assay of MET and TEL in different commercially available formulations.

CONCLUSION

The proposed RP-HPLC - PDA method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of MET and TEL in combination using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of MET and TEL without interference from diluent and placebo. The proposed method is highly sensitive, reproducible, reliable, rapid and specific and also has the unique advantage of LC conditions being compatible with MS detection. Therefore, this method can be employed in quality control to estimate the amount of MET and TEL in bulk and in combined dosage forms.

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