

Research Article



Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Trimetazidine Hydrochloride and Metoprolol Succinate in Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid, precise and accurate spectrophotometric method has been developed for determination of Trimetazidine Hydrochloride and Metoprolol Succinate by absorbance ratio method in combined dosage form. The absorbance ratio method is based on measurement of absorbance at 249.5 nm (isoabsorptive point) and 274 nm (λ max of Metoprolol Succinate) as two wavelengths selected for quantification of Trimetazidine Hydrochloride and Metoprolol Succinate. Method obeyed Beer's law in the concentration range of 40-200 μ g/ml for Trimetazidine Hydrochloride and 54-270 μ g/ml for Metoprolol Succinate. The proposed methods were validated and can be used for analysis of combined dosage tablet formulation containing Trimetazidine Hydrochloride and Metoprolol Succinate.

Keywords: Trimetazidine Hydrochloride, Metoprolol Succinate, Absorbance ratio method, Method development, UV-visible spectrophotometer.

INTRODUCTION

Trimetazidine Hydrochloride, 1-(2,3,4-trimethoxybenzyl)-piperazine hydrochloride, is used in Angina pectoris and is a powerful anti-ischemic agent. The chemical structure of the Trimetazidine Hydrochloride is shown in Fig. 1(a). Trimetazidine hydrochloride usually prescribed as a long-term treatment of angina pectoris¹⁻². Trimetazidine hydrochloride prevents a decrease in intracellular adenosinetriphosphate levels, thereby ensuring the proper functioning of ionic pumps and transmembranous sodium potassium flow whilst maintaining cellular homeostasis.

Metoprolol Succinate, Bis[(2RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl) amino]propan-2-ol] butanedioate is used in the treatment of Hypertension, angina, Acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, congestive heart failure and prevention of migraine headaches. The chemical structure of the Metoprolol Succinate is shown in Fig. 1(b). Metoprolol Succinate competes with adrenergic neurotransmitters such as catecholamines for binding at peripheral (especially cardiac) adrenergic neuron sites, leading to decreased cardiac output¹⁻².

Trimetazidine Hydrochloride is official in IP 2010³ and BP 2009⁴ and Potentiometric titration method is describe for estimation of Trimetazidine Hydrochloride in both IP 2010 and BP 2009. Literature survey reveals that several analytical methods like HPLC method⁵, LC/ESI-MS method⁶, UPLC- MSMS method⁷ and RP-HPLC method⁸ in human plasma and LC- MS/MS method⁹ in rat plasma and spectrophotometric method¹⁰ and HPLC method¹¹ have been reported for the determination of Trimetazidine

Hydrochloride in bulk drug and pharmaceutical formulations.

Metoprolol Succinate is official in BP 2009¹² and USP 2007¹³.

Potentiometric titration method is described for estimation of Metoprolol Succinate in BP 2009 and Liquid chromatographic method in USP 2007.

Literature survey reveals that several analytical methods like spectrophotometric method¹⁴⁻¹⁷, HPLC method¹⁸⁻²², HPTLC method^{23,24} have been reported for the determination of Metoprolol Succinate alone and in combination with other drugs.

Not a single UV method is reported so far for the simultaneous analysis of Trimetazidine Hydrochloride and Metoprolol Succinate in their combined dosage form.

So a need was felt to develop new methods to analyze these drugs simultaneously.

A successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis. In this article simple, rapid, accurate, reproducible and economical methods have been described for the simultaneous determination of Trimetazidine Hydrochloride and Metoprolol Succinate in tablet formulations using Absorbance ratio method.

MATERIALS AND METHODS

Instrumentation

Double beam UV-visible spectrophotometer (Simadzu-1800, Software –UV Probe, Version 2.42) having two matched quartz cells with 1 cm light path. Electronic analytical balance (Mettlertoledo) was used for weighing the materials.



Reagent and Chemicals

Pure samples of Trimetazidine hydrochloride (Gift sample, Triveni Chemical, Vapi) and Metoprolol Succinate (Gift sample, IPCA laboratories) were used in the proposed spectrophotometric analysis.

Combined tablet formulation (Carvidon-MT) was procured from local market.

Methanol AR (Analytical grade) was procured from SD Fine Chemicals, Mumbai.

All the chemicals used were of analytical grade. Distilled water was used as a solvent throughout the study.

Preparation of Stock Solution

Trimetazidine Hydrochloride

Standard stock solution of Trimetazidine was prepared by dissolving 100 mg Trimetazidine in 100 ml of water and sonicated for 20 min and then dilute up to 100 ml to produce a concentration of 1000 µg/ml, which is the standard stock solution.

Metoprolol Succinate

Standard stock solution of Metoprolol Succinate was prepared by dissolving 100 mg of Metoprolol Succinate in 100 ml of water to produce a concentration of 1000 µg/ml, which is the standard stock solution.

Mixture (Marketed tablet)

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder.

The tablet powder equivalent to 100 mg of TMZ and 135 mg of METO was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with water.

The solution was filtered and first few drops of filtrate were discarded.

12 ml of this solution was diluted to 100 ml with water.

Determination of λ_{max}

10 ml of working standard stock solution of TMZ (1000 µg/ml) and 13.5 ml of standard stock solution of METO (1000 µg/ml) were pipette out into two separate 100 ml volumetric flask and volume was adjusted to the mark with water to get 100 µg/ml of TMZ and 135 µg/ml of METO.

Each solution was scanned between 200-400 nm against methanol as a reagent blank.

Wavelengths were selected from the overlay spectra of TMZ and METO.

The optical characteristic and linear regression data is summarized in Table 1 and Fig 2.

Method Development

Absorbance Ratio Method^{25,26}

Two wavelengths selected are 249.5 nm (isoabsorptive point) and 274 nm (λ_{max} of Metoprolol Succinate) for determination of Trimetazidine Hydrochloride and Metoprolol Succinate respectively. 10 ml of working standard stock solution of TMZ (1000 µg/ml) and 13.5 ml of standard stock solution of METO (1000 µg/ml) were pipette out into two separate 100 ml volumetric flask and volume was adjusted to the mark with water to get 100 µg/ml of TMZ and 135 µg/ml of METO. Each solution was scanned between 200 - 400 nm against methanol as a reagent blank. Wavelengths were selected from the overlay spectra of TMZ and METO.

Concentration of the drug in the samples was obtained using the following equation:

$$C_x = \left(\frac{Q_m - Q_y}{Q_x - Q_y} \right) \times \frac{A_1}{a_{x1}}$$

$$C_y = \left(\frac{Q_m - Q_x}{Q_y - Q_x} \right) \times \frac{A_1}{a_{y1}}$$

Where, Q_m= Abs of sample at 274nm (A₂) / Abs of sample at 249.5nm (A₁)

Q_x= Absorptivity of TMZ at 274nm / Absorptivity of TMZ at 249.5nm

Q_y=Absorptivity of METO at 274nm / Absorptivity of METO at 249.5nm

Q_x and Q_y are value of TMZ and METO respectively, a_{x1} and a_{y1} are absorptivity values at isobestic point for TMZ and METO. A₁ and A₂ are absorbance of mixture at λ₁ and λ₂ respectively, C_x and C_y are the concentrations of TMZ and METO in µg/ml respectively.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy, Precision, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ) as per the ICH (International Conference on Harmonization).^{27,28}

Linearity

Various aliquots were prepared from the stock solution (1000µg/ml) ranging from 40- 270 µg/ml. The samples were scanned in UV-VIS Spectrophotometer using Distilled Water as blank. It was found that the selected drug TMZ shows linearity between the 40-200 µg/ml and METO shows between the 54-270 µg/ml. Linear regression data is summarized in Table 1, calibration curve shown in Fig. 3.



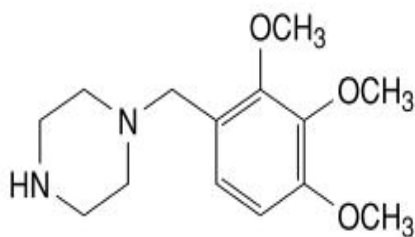


Figure 1(a): Chemical Structure of Trimetazidine Hydrochloride

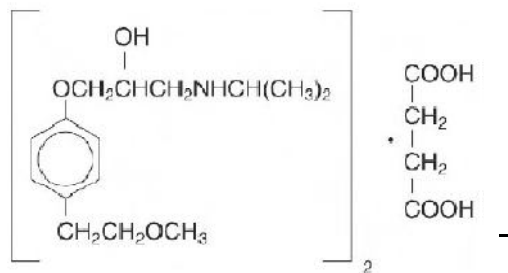


Figure 1 (b): Chemical structure of Metoprolol succinate

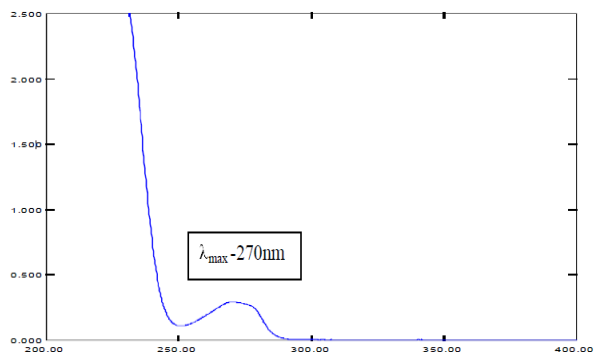


Figure 2(a): UV spectra of standard Trimetazidine (100ppm)

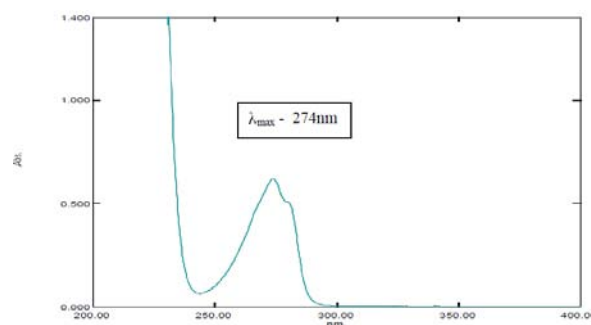


Figure 2(b): UV spectra of standard Metoprolol Succinate (135ppm)

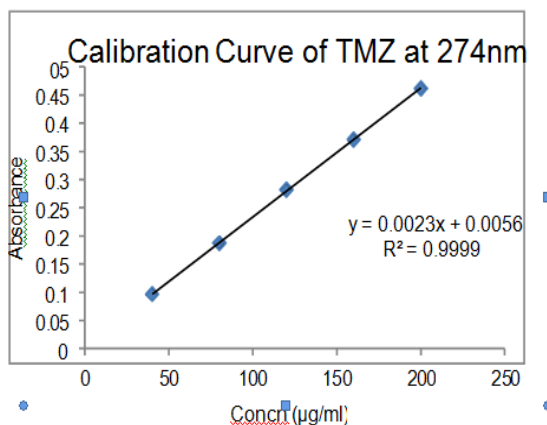


Figure 3(a): Calibration curve for TMZ at 274 nm in Water

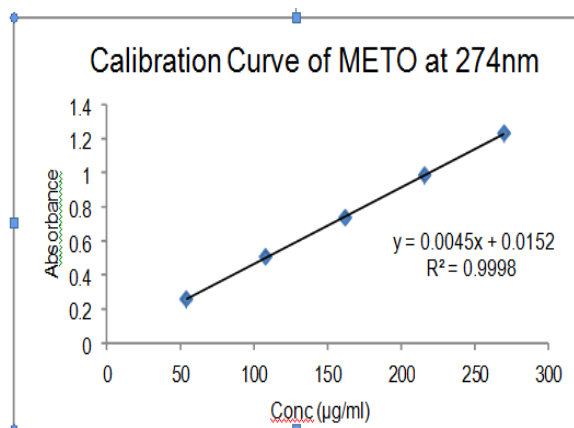


Figure 3(b): Calibration curve for METO at 274 nm in Water

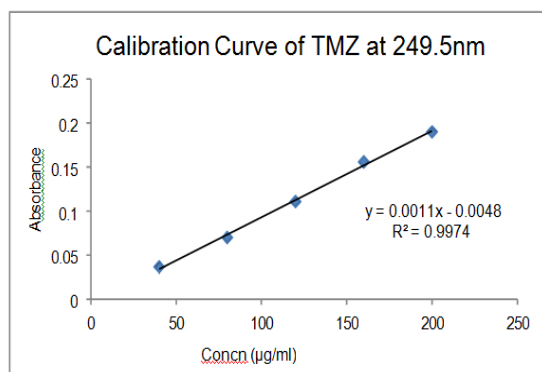


Figure 3(c): Calibration curve for METO at 249.5 nm in Water

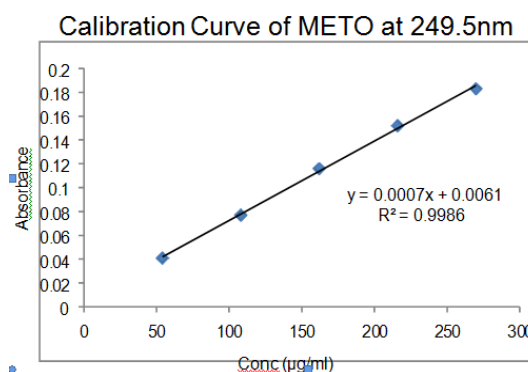


Figure 3(d): Calibration curve for METO at 249.5 nm in Water

Table 1: Summary of optical characteristic and method validation result of Trimetazidine Hydrochloride and Metoprolol Succinate

Parameters	UV Spectrophotometrics				
	λ (nm)	(Isoabsorptive point for TMZ and METO) 249.5 nm		METO(λ max) 274 nm	
		TMZ	METO	TMZ	METO
Beer's Law limits ($\mu\text{g/ml}$)	40-200	54-270	40-200	54-270	
Regression equation (*Y)	0.0011x + 0.0048	0.0007x + 0.0061	0.0023x + 0.0056	0.0045x + 0.0152	
Slope (m)	0.0011	0.0007	0.0023	0.0045	
Intercept (c)	0.0048	0.0061	0.0056	0.0152	
Standard deviation	0.112 \pm 0.00753	0.115 \pm 0.000894	0.281 \pm 0.001378	0.735 \pm 0.001673	
Correlation co-efficient (r^2)	0.9974	0.9986	0.9999	0.9998	
Accuracy					
Level of % recovery	% recovery of drugs at 249.5 nm		% recovery of drugs at 274 nm		
	TMZ	METO	TMZ	METO	
80	99.43	99.67	101.09	99.35	
100	101.62	100.51	100.5	100.51	
120	100.38	98.89	98.28	101.28	
Precision	% RSD for Intraday Precision at 249.5 nm		% RSD for Interday Precision at 274 nm		
	0.668	0.672	0.569	0.428	
	1.578	1.685	1.548	1.420	
LOD ($\mu\text{g/ml}$)	4.29	8.01	2.15	11	
LOQ ($\mu\text{g/ml}$)	13	24.48	6.52	33.33	

Table 2: Analysis data of Marketed Formulation

Tablet batch No.	Label claim($\mu\text{g/ml}$)		Amount found($\mu\text{g/ml}$)		% of label claim estimated	
	Trimetazidine Hydrochloride	Metoprolol Succinate	Trimetazidine Hydrochloride	Metoprolol Succinate	Trimetazidine Hydrochloride	Metoprolol Succinate
CDMY0002	120	162	121.74 \pm 0.203	164.27 \pm 0.805	101.45	101.45
CDMY0001	120	162	118.25 \pm 0.357	162.6 \pm 0.543	98.54	100.37

Precision

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 120 $\mu\text{g/ml}$ for TMZ and 160 $\mu\text{g/ml}$ for METO were prepared and analyzed three times in a day i.e. morning, afternoon and evening and the absorbance were noted.

In the interday variation study, solutions of same concentration 120 $\mu\text{g/ml}$ for TMZ and 160 $\mu\text{g/ml}$ for METO were prepared and analyzed three times for three consecutive days and the absorbances were noted. % RSD is shown in Table 1.

Accuracy

The accuracy of an analytical procedure express the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found.

The accuracy of the method were determined by preparing solutions of different concentrations that is

80%, 100% and 120% in which the amount of marketed formulation was kept constant i.e. 100% and the amount of pure drug of TMZ and METO was added for 80%, 100% and 120% in marketed formulation respectively.

The solutions were prepared and the accuracy was indicated by % recovery. The recovery values for TMZ and METO ranged from 98% to 102% shown in Table 1.

Limit of Detection (LOD)

The Limit of Detection (LOD) was determined based on standard deviation of response of the calibration curve.

The standard deviation of absorbance of calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOD. The result was shown in Table 1.

$$LOD = 3.3 \times \frac{S.D}{S}$$

Where; S.D=standard deviation

S=slope of absorbance of calibration curve

Limit of Quantification (LOQ)

The Limit of Quantification (LOQ) was determined based on standard deviation of response of the calibration curve.

The standard deviation of absorbance of calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOQ. The result was shown in Table 1.

$$LOQ = 10 \times \frac{S.D}{S}$$

Where; S.D=standard deviation

S=Slope of absorbance of calibration curve

Assay of Marketed Formulation (Tablet)

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 100 mg and 135 mg of TMZ and METO respectively and transferred into 100 ml volumetric flask, 12 ml of this solution was diluted to 100 ml with water volume was adjusted to mark with same solvent up to 100 ml to produce a concentration 120 µg/ml of TMZ and 162 µg/ml of METO. Calculated amount found (mg/ml) and % of label claim estimated of TMZ and METO in marketed formulation. The result was shown in Table 2.

RESULTS AND DISCUSSION

The proposed method of analysis for TMZ and METO in combination were validated as per the recommendations of ICH guidelines for parameter like accuracy, precision, linearity, and range, limit of detection and limit of quantification. The drugs obeys Beer's law in concentration range of 40-200 µg/ml for TMZ and 54-270 µg/ml for METO µg/ml with correlation coefficient of 0.999.

Precision of both the methods were calculated by intraday and inter day variation study and %RSD of observation were found to be less than 2, shown in Table 1. Results of recovery studies were within 98-102%, shown in Table 1.

CONCLUSION

A simple, accurate, precise, robust and rapid UV visible spectrophotometric method has been developed for simultaneous estimation of TMZ and METO in pharmaceutical dosage form. The method has been validated for TMZ and METO in pharmaceutical dosage form. The method developed for quantitative determination of TMZ and METO is rapid, precise, accurate and selective. The results of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of TMZ and METO in bulk and pharmaceutical formulation. The developed method can be conveniently used for the assay determination of in

bulk TMZ and METO drugs and pharmaceutical dosage form.

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