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Research Article

Development and Validation of an RP-HPLC Method for Simultaneous Determination of Trimetazidine Hydrochloride and Metoprolol Succinate

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ABSTRACT

An RP-HPLC method has been developed and validated for the simultaneous determination of Trimetazidine Hydrochloride (TMZ) and Metoprolol Succinate (METO) in Tablets. In HPLC method, Enable C18 (250 x 4.6 mm, 5 µm) column was used as stationary phase and Water: Methanol: Acetonitrile in the ratio 45:45:10, (v/v/v) (pH adjusted to 3.0 with ortho- phosphoric acid) as mobile phase was used. The flow rate was 1ml/min and both drugs were quantified at 274.0 nm. The retention time for TMZ and METO was found to be 2.61 ± 0.175 min and 4.12 ± 0.129 respectively. The method was validated according to ICH guidelines for various parameters like accuracy, precision, specificity, linearity, robustness, LOD and LOQ. The linearity of the proposed method was investigated in the range of 20-120 µg/mL (r^2=0.9977) for TMZ and 27 -162µg/mL (r^2 = 0.9970) for METO. The limits of detection (LOD) were 1.205 µg/mL and 1.53 µg/mL for TMZ and METO, and the limits of quantitation (LOQ) were 3.652 µg/mL and 4.64 µg/mL, respectively. The obtained results proved that the method can be employed for the routine analysis of Trimetazidine Hydrochloride and Metoprolol Succinate in bulks as well as in the commercial formulations.

Keywords: RP-HPLC; Trimetazidine; Metoprolol Succinate; Method Development; ICH guidelines; Validation.

1. 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. Trimetazidine usually prescribed as a long-term treatment of angina pectoris[1-2].Trimetazidine prevents a decrease in intracellular adenosinetri phosphate levels, thereby ensuring the proper functioning of ionic pumps and transmembranous sodium potassium flow whilst maintaining cellular homeostasis. Trimetazidine Hydrochloride is official in IP 2010[3] and BP 2009[4] 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 reported for the determination of Trimetazidine Hydrochloride are HPLC method[5] , LC/ESI-MS method[6], UPLC- MSMS method[7] and RP-HPLC method[8] in human plasma and LC- MS/MS method[9] in rat plasma and spectrophotometric method[10] and HPLC method[11] in bulk drug and pharmaceutical formulations.

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. 2. Metoprolol competes with adrenergic

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neurotransmitters such as catecholamines for binding at peripheral (especially cardiac) adrenergic neuron sites,

leading to decreased cardiac output. Metoprolol succinate is official in BP 2009[12] and USP 2007[13]. Potentiometric titration method is describe for estimation of Metoprolol succinate in BP 2009 and Liquid chromatographic method in USP 2007. Literature survey reveals that several analytical methods reported for the determination of Metoprolol succinate alone are spectrophotometric method[14] and HPLC method[15] in bulk drug and pharmaceutical formulations, RP-HPLC method[16] ,Stability indicating reverse phase liquid chromatographic method[17], HPTLC method for simultaneous estimation of Hydrochlorthiazide and

Metoprolol succinate in tablet and bulk dosage[18], HPLC method for simultaneous estimation of Metoprolol succinate and Hydrochlorothiazide in a tablet formulation [19]. RP-HPLC for simultaneous estimation of metoprolol succinate and hydrochlorothiazide[20]. Absorption Correction Method for simultaneous estimation Metoprolol Succinate and Olmesartan Medoxomil in Combined Tablet Dosage Form[21], Spectrophotometric Method for simultaneous estimation of Succinate and Olmesartan Medoxomil in Tablet[22], HPTLC method for simultaneous estimation of Succinate and Olmesartan Medoxomil in Tablet [23], Spectrophotometric method for simultaneous estimation of Metoprolol Succinate and Telmisartan in Tablet[24]. This method is very simple in application in comparison with the previously reported methods and at the same time it offers a high degree of accuracy and precision.

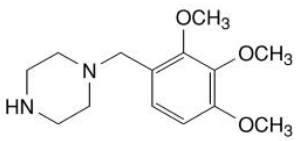

Fig.1: Chemical Structure of Trimetazidine Hydrochloride

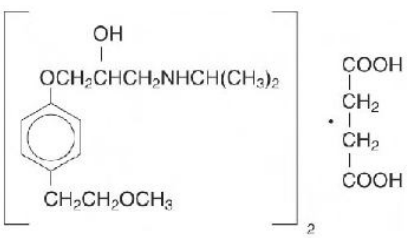

Fig.2: Chemical structure of Metoprolol succinate

2. MATERIALS AND METHODS

2.1 Materials

Standard bulk drug samples of Trimetazidine Hydrochloride and Metoprolol Succinate were provided by IPCA Pharmaceutical Ltd., Gujarat and Triveni Pharmaceutical Ltd., Gujarat, India respectively. Tablets of combined dosage form were procured from the local market.

HPLC grade Methanol and HPLC grade Acetonitrile were obtained form (Merck Ltd., Mumbai, India) . AR grade ortho-phosphoric acid was obtained form (S.D Fine Chemicals Ltd, Mumbai, India)

2.2 Instrumentation

A HPLC instrument (SHIMADZU LC-2010C HT) equipped with a UV-Visible detector and manual injector with 20 µL loop, Enable C18 column (250 mm × 4.6 mm id, 5 µm particle size) and LC solution software were used. All solutions used in HPLC analysis were filtered using a 0.45µm nylon membrane filtration apparatus with vacuum pump. Ultrasonic bath sonicator was used for degassing the mobile phase.

2.3 Chromatographic Conditions

Mobile Phase selected for this method Water: Methanol: Acetonitril (45:45:10v/v, pH adjusted to 3.0 with 0-phosphoric acid). Flow rate employed was 1.0 mL/min. The injection volume was 10 µL and the run time was 10 min. Detection was carried out at 274 nm.

2.4 Preparation of Standard Stock Solution

Accurately weighed 100 mg METO and TMZ were transferred into 100 mL volumetric flasks

and dissolved in mobile phase and made up to the mark to give a stock solution having strength 1000µg/mL of METO and TMZ resepectively.

2.5 Preparation of Working Standard Solutions

Working standard solutions for HPLC injections were prepared on a daily basis Aliquots of the standard stock solution were taken and diluted with the mobile phase to get solutions in a concentration range of 20 -120µg/mL of TMZ and 27-162 µg/mL of METO.

2.6 Assay of Tablet Formulation

20 tablets were accurately weighed and triturated with glass mortar and pestle. The powder equivalent to 100 mg of TMZ and 135 mg of METO was taken in 100 mL volumetric flask; mobile phase was added and the flask was kept in an ultrasonic bath for 10 min. The volume was made up to mark with mobile phase. From this 4 mL was transferred to 10mL volumetric flask and volume was made upto mark with mobile phase. Solution was then filtered through 0.45µL membrane filter. The diluted solution was analyzed under optimized chromatographic conditions. The areas of resulting peak were measured at 274 nm.

2.7 Method Validation

The method was validated as per ICH guidelines to demonstrate that it is suitable for the intended purpose. The method was validated for system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness [25, 26].

2.8 System Suitability

System suitability parameters were studied to ensure that the instrument is suitable for the intended purpose. Retention time, tailing factor and theoretical plates were evaluated. The drug solution was injected five times into chromatographic system under the optimized conditions and the parameters were evaluated.

2.9 Linearity

Aliquots of standard solutions of TMZ and METO in range 20-120 g/mL and 27-162 g/mL respectively, were prepared from working solution and 10µL of each of these solutions was then injected into the column and the chromatographic characteristics were studied under the optimized conditions.

2.10 Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (80, 100 and 120%) and the percentage recoveries were calculated.

2.11 Precision

The precision of the method was determined by intra and inter day precision studies. This was evaluated by injecting three different sample preparations of TMZ and MET from a single formulation at three different concentration levels on the same day (Intraday) and on three different days (Inter day). From the resulting data the % Relative standard deviation was calculate.

2.13 Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

2.14 Robustness

Robustness was established by introducing small deliberate changes in the HPLC optimized conditions which include the change in wavelength, flow rate and percentage of methanol in mobile phase. This was studied using three replicates at a concentration level of 10µg/mL.

3. RESULTS AND DISCUSSION

A simple RP-HPLC method has been developed for determination of Trimetazidine Hydrochloride and Metoprolol Succinate. The method was optimized to provide a good separation of the components (acceptable theoretical plates) with a sufficient sensitivity and suitable peak symmetry in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The chromatographic separation was achieved using an RP C18 column. Our experiments using Water : Methanol: Acetonitril (45:45:10v/v, pH adjusted to 3.0 with o-phosphoric acid) as mobile phase gave a good peak shape (peak symmetry) and resolution for Trimetazidine Hydrochloride and Metoprolol Succinate. The optimized chromatographic conditions are given in Table - 1. The representative chromatogram of standard Trimetazidine Hydrochloride and Metoprolol Succinate are shown in Fig. 3.

The chromatogram for the assay of marketed tablets showed two peaks for Trimetazidine Hydrochloride and Metoprolol Succinate at a retention time of 2.61 min and 4.12 respectively The representative chromatogram is depicted in Fig. 4.

The report for the assay of Trimetazidine Hydrochloride and Metoprolol Succinate tablets is presented in Table -2.

The proposed method was found to be simple. The linearity data is tabulated in Table – 3 and 4. Calibration curve of peak area against concentration was found to be linear in the

concentration range of 20-120 µg/mL and 27-162 µg/mL for TMZ and METO respectively as shown in Fig. 5 and 6 with the regression equation y = 28,543.0476x - 98 162.6667 and y = 32,684.5314x - 178,256.5333 and the correlation coefficient of 0.9970 and 0.9977 for TMZ and METO respectively .

System suitability parameters indicate high column efficiency with large number of theoretical plates (>2000). The tailing factor was found to be 1.20±0.746 for TMZ and 1.43±0.976 for METO which does not exceed the critical value (2). The average retention time was found to be 2.61±0.175 min and 4.12±0.129 min for TMZ and METO respectively. No interference was seen from any of the excipients of the marketed tablet of Trimetazidine Hydrochloride and Metoprolol Succinate indicating the specificity of the method. The results of recovery studies are tabulated in Table – 8 and 9. Good recovery of the spiked drug was obtained at each added concentration.

The data for precision is represented in Table – 5,6 and 7. The %RSD was found to be 1.226-1.425 and 1.142-1.242 for Trimetazidine Hydrochloride and Metoprolol Succinate respectively for intraday and 1.903-1.860 and1.861-1.956 for Trimetazidine Hydrochloride and Metoprolol Succinate respectively for inter day precision studies. Thus the developed method was found to be accurate and precise as the % RSD value was less than 2.

The limit of detection for Trimetazidine Hydrochloride and Metoprolol Succinate were found to be 1.205 µg/mL and 1.53 µg/mL respectively and limit of quantification for Trimetazidine Hydrochloride and Metoprolol Succinate were found to be 3.652 µg/mL and 4.64 µg/mL respectively. The results of robustness study are given in Table – 10 and11. It was found that there was no drastic change in the resolution of Trimetazidine Hydrochloride and Metoprolol Succinate when deliberate changes were introduced in the optimized chromatographic conditions thus confirming robustness of the developed method.

Table 1: Optimized chromatographic conditions

Parameters	Optimized conditions
HPLC system	SHIMADJU LC-2010C HT
Column	Enable C18 (250x 4.6mm,5 µm)
Mobile phase	Water: Methanol: Acetronitril (45:45:10v/v pH adjusted to 3.0 with orthophosphoric acid).
Flow rate	1.0 ml/min
Detection wavelength	274 nm
Injection volume	10.0 µl
Concentration of Standard Trimetazidine Hydrochloride and Metoprolol Succinate	TMZ (80ppm) and METO (108ppm

Table 2: Report for assay of Trimetazidine Hydrochloride and Metoprolol Succinate

Drug Formulation	Amount present µg/ml		Amount Found µg/ml		% Labelled Claim	
	TMZ	METO	TMZ	METO	TMZ	METO
CDMY0001	80	108	79.29±0.167	108.44±0.091	99.12	100.4
CDMY0002	80	108	80.6±0.216	109.2±0.165	100.75	101.11

Table 3: Linearity data for TMZ

Concentration (µg/ml)	Mean area ± S.D. (n=3)
20	474637 ± 2132.11
40	1180911 ± 21885.23
60	1751308 ± 19897.56
80	2345561 ± 34087.32
100	3163449 ± 33934.95
120	3742098±16838.34

Table 4: Linearity data for METO

Concentration (µg/ml)	Mean area ± S.D. (n=3)
27	598314 ± 270.045
54	1493194 ±2696.33
81	2236120 ± 20325.49
108	2982183 ± 16990.12
135	3863668 ± 31479.9
162	4421453±30128.81

Table 5: Repeatability data for TMZ and METO

Conc of TMZ	Area of TMZ	Conc of METO	Area of METO
1	2338652	**1**	2978563
2	2347569	**2**	3981452
3	2350236	**3**	2983653
4	2348632	**4**	2985526
5	2353625	**5**	2975655
6	2335632	**6**	2982562
S.D	20032.48	**S.D**	21822.7
%RSD	0.854	**%RSD**	0.732

Table 6: Intraday precision data of TMZ and METO

Drug	Concentration (µg/mL)	Intraday precision	
		Mean area ± S.D	% RSD
Trimetazidine	40	1179786±14464.17	1.226
	60	1750046±16975.4	0.970
	80	2344695±33411.90	1.425
Metoprolol Succinate	54	1490127±17011.25	1.142
	81	2235334±34088.84	1.525
	108	2981631±37031.85	1.242

Table 7: Interday precision data of TMZ and METO

Drug	Concentration (µg/mL)	Interday precision	
		Mean area ± S.D	% RSD
Trimetazidine	40	1176694±22401.56	1.903
	60	1744393±34695.97	1.989
	80	2339861±43630.35	1.860
Metoprolol Succinate	54	1488761±27707.79	1.861
	81	2234322±36701.13	1.642
	108	2278866±44574.61	1.956

Table 8: Recovery data for TMZ

Conc. of TMZ in tablet (µg/mL)	Conc. of std. TMZ spiked	Total Conc. of TMZ	Mean area ± S.D. (n=3)	Conc. Recovered	% Recovery
60	0	60	1757251±909.61	-	-
60	48	108	3335327.2±11094.0	107.5	99.53
60	60	120	3792910.2±23605.35	121.5	101.25
60	72	132	4080533.8±16315.81	130.3	98.71

Table 9: Recovery data for METO

Conc. of TMZ in tablet (µg/mL)	Conc. of std. TMZ spiked	Total Conc. of TMZ	Mean area ± S.D. (n=3)	Conc. Recovered	% Recovery
81	0	81	2243751±6286.5	-	-
81	64	145	4094804.7±10875.9	146.9	101.31
81	81	162	4560055±21909.8	163.2	100.74
81	98	179	4979637.1±172682.14	177.9	99.38

Table 10: Results of Robustness Study for TMZ

Parameters	Mean area ± S.D.	%RSD
Flow rate +0. 2	2250343±19154.57	0.851
Flow rate – 0.2	2379604 ± 29303.79	1.231
Mobile phase + 2	2273358 ± 11104.57	0.488
Mobile phase – 2	2287528 ± 10158.97	0.444
pH + 0.2	2322860 ± 20899.25	0.899
pH –0. 2	2367047 ± 16492.84	0.696

Table 11: Results of Robustness study for METO

Parameters	Mean area ± S.D.	%RSD
Flow rate +0. 2	2950332 ± 46684.18	1.582
Flow rate – 0.2	3037950 ± 28940.72	0.952
Mobile phase + 2	2973238 ± 7489.36	0.251
Mobile phase – 2	2983548 ± 16166.72	0.541
pH + 0.2	2966657 ± 13947.65	0.470
pH – 0.2	2992533 ± 15102.61	0.504

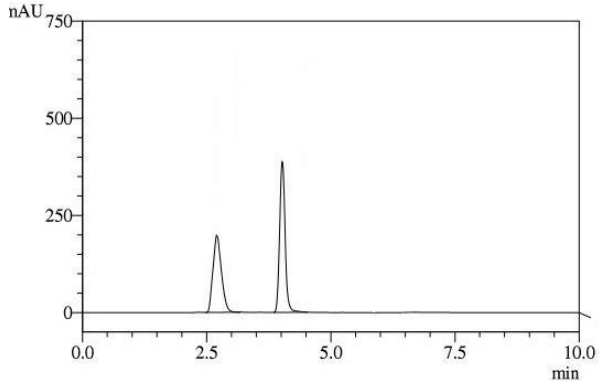

Fig. 3: Chromatogram of standard TMZ (80ppm) and METO (108ppm)

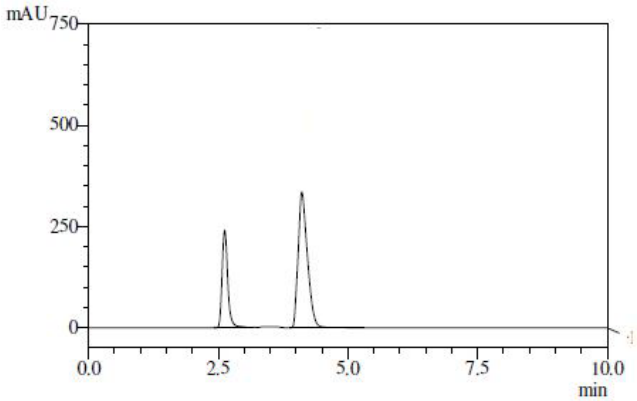

Fig.4: Representative chromatogram of Trimetazidine Hydrochloride and Metoprolol Succinate in tablet formulation.

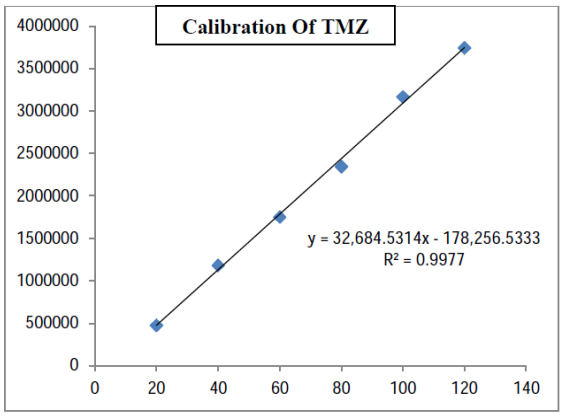

Fig.5: Calibration curve for TMZ.

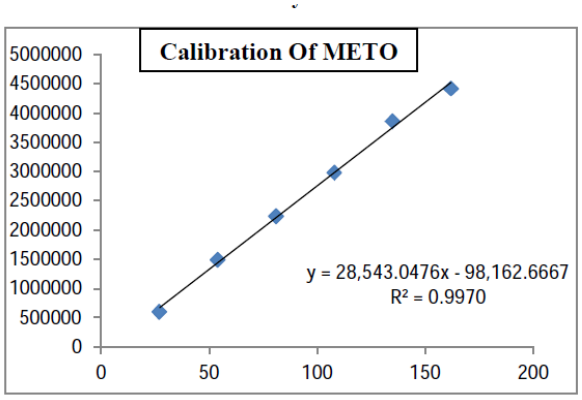

Fig.6: Calibration curve for METO

4. CONCLUSION

A validated RP-HPLC analytical method has been developed for the determination of Trimetazidine Hydrochloride and Metoprolol Succinate in bulk and pharmaceutical dosage form. The proposed method is accurate, precise, specific and suitable to use for the routine analysis of Trimetazidine Hydrochloride and Metoprolol Succinate in either bulk API powder or pharmaceutical dosage forms.

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