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Determination of metoprolol tartrate and hydrochlorothiazide in tablet dosage form by high performance liquid chromatography

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ABSTRACT

A simple and rapid reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for simultaneous determination of metoprolol tartrate (MET) and hydrochlorothiazide (HTZ) in tablet dosage form. The chromatography was carried out on a C 18 (250 mm x 4.6 mm, 10 µm) column. The components were separated isocratically with a mobile phase consisting of 35 volumes 0.5 % v/v orthophosphoric acid, 15 volumes of methanol and 50 volumes of acetonitrile at a flow rate of 1.0 ml/min. The UV detector was set at 280 nm. The retention times for MET and HTZ were found to be 3.19 min and 8.37 min, respectively. The method was validated for the parameters like specificity, linearity, precision, accuracy, limit of quantitation and limit of detection. The method was found to be specific as no other peaks of impurities and excipients were observed. The square of correlation coefficients (R^2) for MET and HTZ were 0.9999 and 0.9985 while percentage mean recoveries were 99.76 % and 99.56 %, respectively. Intra- and inter-day relative standard deviations for both the components were <2.0%. The proposed RP-LC method can be applied for the routine analysis of commercially available formulations of these drugs either as such or in combination.

Key Words: liquid chromatography, validation, metoprolol tartrate, hydrochlorothiazide, tablet dosage form

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INTRODUCTION

Clinical trials demonstrate that most hypertensive patients will not achieve goal blood pressure with a single drug alone, further supporting the use of multi drug therapy [1]. Combining drugs with complementary mechanisms of action permits use of lower doses of each, reducing the risk of dose-dependent adverse reactions. Combinations of beta-blockers and diuretics, usually hydrothiazide class, are very common in antihypertensive therapy [2]. Metoprolol and Hydrochlorothiazide are widely used in the treatment of hypertension, cardiac and renal diseases. Chemically, Metoprolol is 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol and Hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide. Combination is official in the United States Pharmacopoeia which recommended liquid chromatographic assay with two different chromatographic conditions for each of drugs [3]. Various methods have been reported in the literature for simultaneous determination of MET and HTZ in tablet dosage forms which includes spectrophotometry [4-9], high performance liquid chromatography [8-15] and electrophoresis [16].

The aim of the present study was to develop and validate a HPLC method for the simultaneous determination of MET and HTZ in tablet dosage form contained 50 mg MET and 12.5 mg HTZ. The method described complied with

validation requirements of ICH and could be successfully applied for routine quality control of pharmaceutical formulations.

EXPERIMENTAL SECTION

Reagents and chemicals

Tablets, each containing 50 mg MET and 12.5 mg HTZ, were supplied commercially. Metoprolol tartrate RS and Hydrochlorothiazide RS were used as standards and were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and acetonitril were used to prepare the mobile phase. All other chemicals used for the chromatographic experiments were of a reagent grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a modular HPLC system LC-10AShimadzu (Japan) comprising a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 µl loop, column oven CTO-10A, SPD-M10A UV detector with fixed wavelength and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C18, 250 mm x 4.6 mm, 10 µm column eluted with a mixture of 0.5 % v/v orthophosphoric acid, methanol and acetonitrile (35:15:50 *v/v/v*) as the mobile phase at flow rate of 1 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. Detection was carried out by absorbance at 280 nm. The analysis was carried out at an ambient temperature and injection volume was 20 µl.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving 50.0 mg of accurately weighed Metoprolol tartrate RS and 12.5 mg Hydrochlorothiazide RS in methanol, in a 100.0 mL volumetric flask. Reference solution (b): The solution was prepared by diluting 5.0 mL of reference solution (a) with methanol into a 25.0 mL volumetric flask.

Sample preparation

The homogenized powder from twenty tablets with an average weight equivalent to 50 mg MET and 12.5 mg HTZ was transferred into a 100.0 mL volumetric flask. Approximately 70 mL methanol was added and the obtained mixture was mechanically shaked for 20 min. The content was diluted to volume with methanol to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 5.0 mL of the filtrate was diluted into a 25.0 mL volumetric flask to give a test solution containing 100 µg/mL MET and 25 µg/mL HTZ.

RESULTS AND DISCUSSION

In this work an LC method with UV detection for analysis of MET and HTZ in a tablet formulation was developed and validated. From the chromatogram shown in Figure 1, it is evident that, under the proposed chromatographic conditions, MET and HTZ are completely separated, which indicates that the method is selective and could be applied for their simultaneous identification and quantification.

Method validation

The proposed method was validated as per ICH guidelines [17] with respect to specificity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Specificity

The specificity of the method was determined by checking the interference of the components against placebo. No interference was observed for any of the excipients of both drugs.

Linearity

Linearity was evaluated by determining five different concentrations of the standard working solutions of MET and HTZ in triplicate. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. Calibration plot data slope (a), intrercept (b), and correlation coefficients (R^2) were listed in table 1.

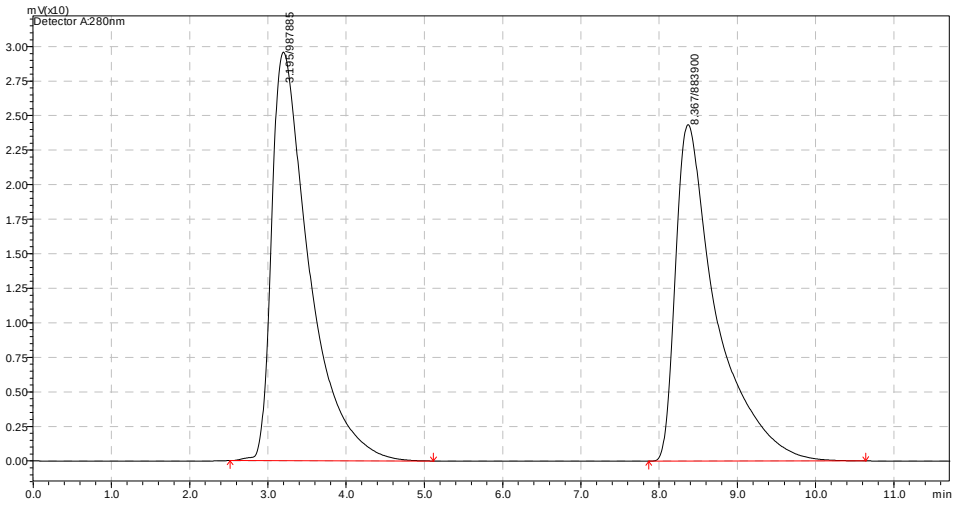

Figure 1: Chromatogram of Metoprolol tartrate RS and Hydrochlorothiazide RS

Table 1: Linear regression data for calibration curves

Drugs	MET	HTZ
Concentration range (µg/mL)	25.00-200.0	6.25-50.00
Slope	125412.2	110213.4
Intercept	1450.1	1211.5
Correlation coefficient (R^2)	0.9999	0.9985

Limits of quantitation and limits of detection

The limit of detection (LOD) was calculated to be three times the standard deviation of baseline noise from analysis of each compound. The limit of quantitation (LOQ) was measured as the lowest of analyte that could be reproducibly quantified above the baseline noise, i.e. for which duplicate injection resulted in an RSD ≤ 2%. The LOQs for MET and HTZ were found to be 2 µg/mL and 0.5 µg/mL, while the LODs were 0.4 µg/ml and 0.2 µg/ml, respectively.

Precision

To check precision (percentage RSD) of analytical method, six replicate samples of the same concentrations of MET and HTZ were analysed. The RSD values measured during assessment of intraday and interday precision were <2.0% for both MET and HTZ, confirming the method is precise (table 2).

Table 2: Intra-day and inter-day precision of the method described

Metoprolol tartrate			Hydrochlorothiazide		
Amount claimed (mg/tablet)	Amount found (mg/tablet)		Amount claimed (mg/tablet)	Amount found (mg/tablet)	
	Intra-day repeatability	Inter-day repeatability		Intra-day repeatability	Inter-day repeatability
50.00	49.54	50.13	12.50	12.51	12.08
	49.85	49.25		12.38	12.54
	50.34	49.74		12.42	12.41
	49.27	49.51		12.20	12.38
	49.44	50.09		12.68	12.64
	49.30	50.21		12.57	12.27
Mean	49.62	49.82	Mean	12.46	12.39
SD	0.409	0.387	SD	0.166	0.198
%RSD	0.82	0.78	%RSD	1.332	1.598

Accuracy

Accuracy was studied by adding three different amounts (corresponding to 50, 100 and 150 % of the test preparation concentrations) of MET and HTZ to the placebo preparation and comparing the actual and measured concentrations.

For each level, three solutions were prepared and recovery of MET and HTZ from placebo was determined. The results from study of accuracy were presented in table 3.

Table 3: Accuracy of the HPLC method

Drug	Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	Mean recovery (%) ± SD	RSD (%)
Metoprolol tartrate	50	50.07	50.01	99.49±0.488	0.49
			49.89		
			49.54		
	100	99.12	99.01	99.71±0.201	0.20
			98.85		
			98.62		
	150	149.23	149.5	100.1±0.197	0.20
			149.1		
			149.7		
Hydrochlorothiazide	50	12.08	12.23	99.24±1.705	1.72
			11.85		
			11.89		
	100	25.32	25.24	99.66±0.645	0.65
			25.40		
			25.07		
	150	37.61	37.30	99.79±0.729	0.73
			37.82		
			37.46		

CONCLUSION

The newly developed LC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation containing metoprolol tartrate and hydrochlorothiazide.

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