

Research Article

Development of Reverse-Phase HPLC Method for Simultaneous Analysis of Metoprolol Succinate and Hydrochlorothiazide in a Tablet Formulation

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Abstract

Purpose: To develop a simple, sensitive and rapid reverse phase HPLC method for the simultaneous analysis of metoprolol succinate and hydrochlorothiazide in a solid dosage form.

Methods: The drugs were analysed by a reverse phase C-18 column using 50mM di-sodium hydrogen phosphate:methanol:acetonitrile in a ratio of 525:225:250 as mobile phase. The flow rate was 1 ml/min and the compounds were detected by a UV-detector at 222 nm at a column temperature of 24 ± 2 °C. The method was statistically validated for linearity and accuracy.

Results: The retention time and drug content of metoprolol succinate and hydrochlorothiazide were 5.38 min, 96.05 % and 3.04 min., 97.64 %, respectively.

Conclusion: The study shows that the developed method is simple and accurate and that it would be suitable for the simultaneous determination of metoprolol succinate and hydrochlorothiazide in pharmaceutical formulations.

Keywords: Metoprolol succinate; Hydrochlorothiazide; Simultaneous analysis; High performance liquid chromatography; Tablet formulation

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INTRODUCTION

Metoprolol succinate is a beta-blocker and while hydrochlorothiazide is a potent thiazide diuretic that enhances natriuresis, leading to reduction in plasma volume and cardiac output. Therefore, it is used widely alone or in combination with other antihypertensive drugs for the treatment of cardiovascular disorders, viz, hypertension, angina, and congestive cardiac failure [1-4]. Chemically, metoprolol succinate is 2-propanol, 1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-, ($\pm$)-, butanedioate and Hydrochlorothiazide is 6-chloro-3,4-dihydro-2H- 1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Both drugs are official in the British Pharmacopoeia and United States Pharmacopoeia but their combination is not and, to the best of our knowledge, no official method has yet been developed for their separation and assay [5,6]. A literature survey revealed spectrophotometric and HPLC methods for the determination of metoprolol succinate and hydrochlorothiazide individually in pharmaceutical dosage forms as well as in biological fluids [7-16]. A new drug application (NDA) has been submitted to FDA (U.S. Food and Drug Administration) by AstraZeneca, a multinational pharmaceutical firm, in respect of the above combination [17]. Thus, application of an HPLC method with high sensitivity and selectivity will find use for the determination of metoprolol succinate and hydrochlorothiazide in pharmaceutical formulations.

EXPERIMENTAL

All solvents were of HPLC grade and reagents were of analytical grade. Acetonitrile and methanol were obtained from Merck®; orthophosphoric acid and di-sodium hydrogen phosphate were purchased from Rankem (Ranbaxy). Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a membrane filter (Millipore Millex®-FH, filter units,

Durapore-PVDF, Polyethylene, 0.22 μ m pore size) and degassed before use.

The active pharmaceutical ingredients (API), metoprolol succinate (assigned purity: 99.1%) and hydrochlorothiazide (assigned purity: 100.0%), were donated by M/s Ajanta Pharma Limited, Mumbai and M/s Ipca Laboratories, Mumbai, India, respectively, and used as reference standards without further purification.

Tablet formulation

Tablets, each containing 95 mg of metoprolol succinate and 12.5 mg of hydrochlorothiazide together with other tablet excipients (lactose - 172 mg; starch - 15 and 35 mg for paste and powder respectively; magnesium stearate - 2 mg and talc - 18 mg) were prepared in the laboratory as follows. Metoprolol succinate, hydrochlorothiazide and lactose were mixed well, and the mixture was moistened uniformly with 10% starch mucilage. Granules were then prepared by passing it through a 1.41 mm aperture sieve and dried in an oven at 60 °C for 45 min. The dried granules were passed through 0.84 mm aperture sieve, and mixed thoroughly with dry starch and talc. Three batches, similar in composition and coded MSHT-1, MSHT-2, and MSHT-3, respectively, were made. Tablets (diameter, 10 mm and thickness, 5 $\pm$ 0.2 mm) were produced from the formulations using a hand operated single punch tablet press (Rolex, Ambala, India)

Instrumentation

Quantitative analysis was performed on isocratic high performance liquid chromatography system (HPLC, Waters, Milford, USA) with two Waters 515 pumps, a fixed wavelength programmable 2487 dual wavelength absorbance detector (Waters, Milford, USA) fixed at 222 nm. A guard column (C-18, Shim-pack, Merck, Germany), reverse phase C-18 column (Lichrospher® Merck, 250 $\times$ 4 mm, 5 μ m particle size) and rheodyne injection valve with 20 μ l loop were

used. The HPLC system was equipped with a software, Millenium® (Waters, Milford, USA).

Chromatographic conditions

All analyses were done at a column temperature of 24 ± 2 °C under isocratic conditions. The mobile phase consisted of a volumetric mixture of aqueous 50mM disodium hydrogen phosphate (pH adjusted to 5.0 with orthophosphoric acid): methanol: acetonitrile (in the ratio of 525:225:250). The flow rate was 1.0 ml/min and volume of injection was 20 µl. UV detection was made at 222 nm.

Preparation of standard

A stock solution of the drugs was prepared by dissolving accurately weighed 95 mg of metoprolol succinate and 12.5 mg of hydrochlorothiazide in a 100 ml volumetric flask containing 20 ml mobile phase, warmed on a water bath for about 15 min and then final volume was raised to 100 ml using the mobile phase. Daily working standard solutions were prepared by suitable dilution of stock solution with mobile phase to 10 ml.

Sample preparation

Mean tablet weight was determined by taking the mean of the weights of 20 individual y tablets. The tablets were then powdered in a mortar with a pestle and an accurately weighed portion, equivalent to 95 mg of metoprolol succinate and 12.5 mg of hydrochlorothiazide, was transferred to a 100 ml volumetric flask containing 20 ml mobile phase. This mixture was shaken well with a mechanical shaker for 30 min and then warmed on a water bath for about 15 min. Extraction was carried out three times with the mobile phase (20 ml each time) and the final volume was raised to 100 ml. Finally, the solution was filtered through a Millipore assembly using 0.22 µ filter and appropriately diluted with mobile phase to 10 ml prior to analysis.

Calibration curves

Five sets of different concentration levels (2.0, 4.0, 8.0, 16.0, 32.0 ppm) were prepared for each standard solution. Each of these drug solutions (20 µl) was injected into the chromatographic system ($n = 3$). The peak area and retention time were recorded, and the mean values of peak area ratio were plotted against concentrations.

Recovery studies were performed by standard addition method. A known concentration of working standard was added to a fixed concentration of the pre-analysed test solution. Recovery (%) was calculated by comparing the area before and after the addition of the working standard. The same approach was used for the two drugs.

RESULTS

Typical chromatograms obtained are shown in Figure 1. The retention time (RT) of hydrochlorothiazide and metoprolol succinate were 3.04 and 5.38 min, respectively. The calibration curve showed linearity over a concentration range of 2 to 32 µg/ml for both drugs, with correlation coefficients of 0.9965 and 0.9878, respectively; regression coefficients (r^2) were 0.9929 and 0.9971, respectively; and representative linear regression equations were $Y = 0.0426x + 0.0713$ and $Y = 0.1273x + 0.045$ for hydrochlorothiazide and metoprolol succinate, respectively.

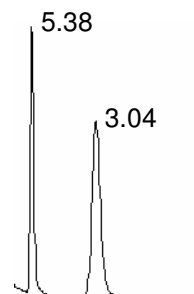


Figure 1: A typical chromatogram of hydrochlorothiazide (RT: 3.04 min) and metoprolol succinate (RT: 5.38 min)

The assay results are given in Table 1. The mean drug content was found to be 99.49 ± 1.32 % for metoprolol succinate and 98.27 ± 0.17 % for hydrochlorothiazide.

The recovery test, which was performed in triplicate, indicate that mean recovery was 97.6 ± 0.5 and 96.1 ± 0.49 % for hydrochlorothiazide and metoprolol succinate, respectively (Table 2).

Table 1: Assay data for combined metoprolol succinate and hydrochlorothiazide (MSHT) formulation

Tablet	Metoprolol succinate			Hydrochlorothiazide		
	Dose (mg)	Content/tab (mg)	% Label	Dose (mg)	Content/tab (mg)	% Label
MSHT-1	95.0	95.7	100.7	12.5	12.5	99.6
MSHT-2	95.0	93.1	97.9	12.5	12.2	97.4
MSHT-3	95.0	94.8	99.8	12.5	12.2	97.8

Table 2: Recovery data for standard solutions added to tablet formulations

Sample name	Amount of drug added (μ g) to powdered tablet formulation	Amount (μ g) found (n = 3)	% Recovery (n = 3)
Metoprolol Succinate	0.5	0.472	94.40
	1.0	0.969	96.90
	1.5	1.453	96.87
Hydrochlorothiazide	0.5	0.463	92.60
	1.0	1.03	103.0
	1.5	1.46	97.33

DISCUSSION

In order to develop an effective method for the analysis of the drugs in pharmaceutical formulations, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection of wavelength, ideal mobile phase and their proportions, optimum pH and concentration of the standard solution were studied. Several binary or ternary eluents were tested using various proportions of solvents including acetonitrile, methanol and di-sodium hydrogen phosphate (50 and 60 mM). The flow rate of 1.0 ml/min for the mobile phase was selected after these preliminary tests.

The development of HPLC methods for the determination of drugs has received

considerable attention over the years because of their reliability in the quality control of drugs and drug products. The goal of this study was to develop a rapid HPLC method for the analysis of metoprolol succinate and hydrochlorothiazide in a finished tablet formulation using a commonly employed reverse phase C-18 column with UV detector. The proposed method is simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in analysis of drugs. The calibration curve showed linearity over a concentration range of 2 to 32 μ g/ml for both drugs and was linear with a correlation coefficient of 0.9965 and 0.9878 for hydrochlorothiazide and metoprolol succinate respectively, and with regression

coefficient (r^2) of 0.9929 and 0.9971, respectively.

Recovery test, which was performed in triplicate, averaged 97.6 ± 0.5 and $96.1 \pm 0.49\%$ for hydrochlorothiazide and metoprolol succinate, respectively, indicating that the proposed method for the analysis of drugs is highly accurate.

CONCLUSION

The results of this study showed that the developed method is simple and accurate. It should be useful for the simultaneous determination of metoprolol succinate and hydrochlorothiazide in pharmaceutical formulations.

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